VOLUME XXXI APRIL, 1952 NUMBER 4

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Contents

The *in Vitro* Metabolism of Estrogens by the Liver Studied by Means of a Colorimetric Method. SEYMOUR LIEBERMAN, HENRY J. TAGNON, AND PHYLLIS SCHULMAN 341

The Metabolism of Estrogens by the Human Liver Studied *in Vitro*. HENRY J. TAGNON, S. LIEBERMAN, PHYLLIS SCHULMAN, AND ALEXANDER BRUNSCHWIG 346

Comparative Effects of a Purified and an Adequate Diet on the Course of Fatty Cirrhosis in the Alcoholic. GERALD B. PHILLIPS, GEORGE J. GABUZDA, JR., AND CHARLES S. DAVIDSON 351

Renal Excretion of Inorganic Phosphate in Newborn Infants. WALLACE W. MCCRORY, CAROLYN W. FORMAN, HELEN MCNAMARA, AND HENRY L. BARNETT 357

The Exchangeable Potassium Content of Normal Women. JERRY K. AIKAWA, GEORGE T. HARRELL, AND BERNARD EISENBERG 367

The Distribution of the Thiosulfate Ion with Respect to Normal Human Serum and Red Blood Cells and its Application to "Extracellular Fluid" Determination. HENRY J. KOWALSKI AND DAVID D. RUTSTEIN .. 370

Amino Acid Studies and Clinical Findings in Normal Adults and Rheumatoid Arthritis Patients Treated with ACTH. A. L. BORDEN, E. C. BRODIE, E. B. WALLRAFF, W. P. HOLBROOK, D. F. HILL, C. A. L. STEPHENS, JR., R. B. JOHNSON, AND A. R. KEMMERER 375

The Lung Volume and its Subdivisions in Normal Boys 10–17 Years of Age. MINERVA MORSE, FREDERIC W. SCHLUTZ, AND DONALD E. CASSELS 380

The Effects of Pitressin on the Metabolism and Excretion of Water and Electrolytes in Normal Subjects and Patients with Cirrhosis and Ascites. W. P. NELSON, III, AND L. G. WELT 392

Diurnal Variation in the Diuretic Response to Ingested Water. SOLOMON PAPPER AND JACK D. ROSENBAUM 401

Potassium and Sodium of Red Blood Cells in Sickle Cell Anemia. D. C. TOSTESON, ETHEL SHEA, AND R. C. DARLING 406

Studies of Total Body Water with Tritium. T. C. PRENTICE, W. SIRI, N. I. BERLIN, G. M. HYDE, R. J. PARSONS, E. E. JOINER, AND J. H. LAWRENCE 412

Postoperative Potassium Deficit and Metabolic Alkalosis. The Pathogenic Significance of Operative Trauma and of Potassium and Phosphorus Deprivation. LEONARD P. ELIEL, OLOF H. PEARSON, AND FREDERICK C. WHITE 419

The Journal of Clinical Investigation

Printed by the Lancaster Press, Inc., Prince and Lemon Sts., Lancaster, Pa. Published by the American Society for Clinical Investigation. Publication Office, c/o Cincinnati General Hospital, Cincinnati 29, Ohio.

EUGENE B. FERRIS, EDITOR-IN-CHIEF

ASSOCIATE EDITORS

WILLIAM B. BEAN RICHARD W. VILTER

The Editorial Committee of the American Society for Clinical Investigation

S. HOWARD ARMSTRONG, JR., *Chicago, Ill.*
STANLEY E. BRADLEY, *New York, N. Y.*
WILLIAM J. DARBY, *Nashville, Tenn.*
LUDWIG W. EICHNA, *New York, N. Y.*
FRANK L. ENGEL, *Durham, N. C.*
JACOB E. FINESINGER, *Baltimore, Md.*
THOMAS HALE HAM, *Cleveland, O.*
FRANZ J. INGELFINGER, *Boston, Mass.*
F. RAYMOND KEATING, JR., *Rochester, Minn.*
JOSEPH L. LILIENTHAL, JR., *Baltimore, Md.*
JOHN A. LUETSCHER, JR., *San Francisco, Calif.*
MACLYN MCCARTY, *New York, N. Y.*
JOHN P. PETERS, *New Haven, Conn.*
WESLEY W. SPINK, *Minneapolis, Minn.*
ROBERT W. WILKINS, *Boston, Mass.*

THE JOURNAL OF CLINICAL INVESTIGATION is designed for the publication of original investigations dealing with or bearing on the problems of disease in man, and it is the policy of the editors that the JOURNAL should cover the field of clinical investigation in its broadest sense.

Contributions should be sent to the **Editor-in-Chief, Dr. Eugene B. Ferris, c/o Cincinnati General Hospital, Cincinnati 29, Ohio.** In order to hasten the process of reviewing and editing it is requested that each original manuscript be accompanied by a duplicate copy.

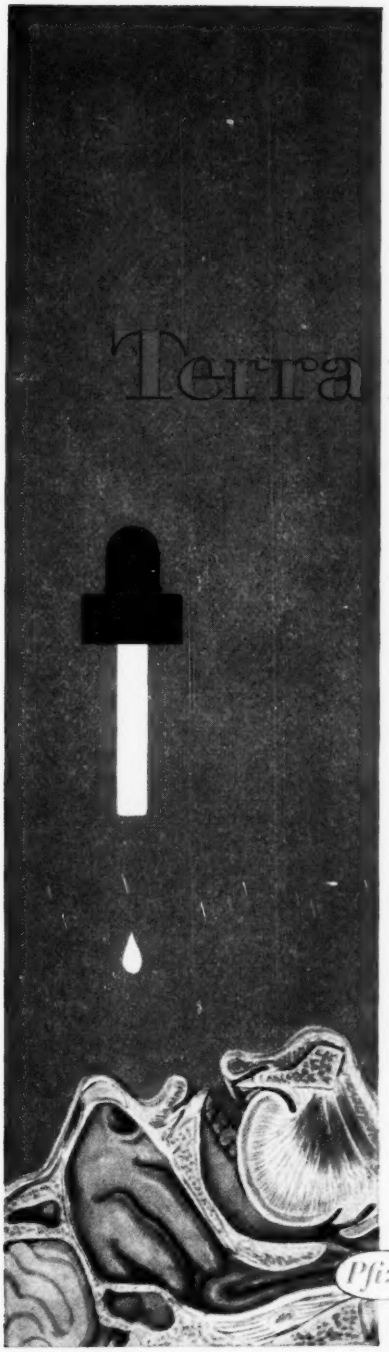
In order further to expedite the earliest possible publication of articles submitted to the JOURNAL OF CLINICAL INVESTIGATION they will be published in the order of final processing after review, correcting and editing rather than in the strict sequence of their receipt in this office. Papers which require much editorial change, revision of charts and tables, condensation and rewriting will be delayed according to the time required for their correction. This policy will put a premium on excellence of manuscript as well as the major consideration of content. Because of the increasing costs of printing, especially in the hand work of making tables, the editors suggest that when feasible photographs of tables be prepared. It is also requested that charts be photographed, the photographs accompanying the manuscript. The originals of the charts need not be forwarded except by special request of the editors. Further information regarding the preparation of manuscripts may be obtained from the editors.

Each volume will consist of 12 monthly issues appearing throughout the calendar year. The subscription price is $12 per volume, **payable in advance**; foreign countries also $12, payable in U. S. funds. Single copies will be sold when available at Vols. 1-28, $2; Vols. 29-31, $1.50 a copy. Authors may purchase reprints at scheduled prices. **A special subscription rate of $6 per volume of 12 monthly issues is offered to medical students, interns, fellows, and residents of approved hospitals.** Subscriptions are **net postpaid.** Remittance should be made by draft, check or postal money order, payable to **The Journal of Clinical Investigation, c/o Cincinnati General Hospital, Cincinnati 29, Ohio.** Claims for copies lost in the mails must be received within **thirty** days (domestic), **ninety** days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

Acceptance for mailing at special rate of postage provided for in paragraph (d-2), section 34.40, P.L. & R. of 1948, authorized February 13, 1950

Entered as second-class matter September 16, 1931, at the Post Office at Lancaster, Pa., under the Act of March 3, 1879

Copyright, 1952, American Society for Clinical Investigation



Terramycin Nasal *(with desoxyephedrine HCl)*

Well tolerated by the nasal mucosa. Terramycin Nasal (with desoxyephedrine HCl) offers both wide-range topical antimicrobial action and mild but prolonged vasoconstrictor effect in the local treatment of acute rhinitis, vasomotor rhinitis and nasopharyngitis. Terramycin Nasal (with desoxyephedrine HCl) may be administered by atomizer, as original drops, or in gauze packs without adversely affecting ciliary action. For best results administer at the earliest symptoms.

the only broad-spectrum antibiotic available as nasal drops

supplied Convenient 5 cc. dropper vials containing 5 mg. Terramycin as the Crystalline Hydrochloride per cc. with 0.25% desoxyephedrine HCl, buffers and aromatics.

Pfizer ***World's Largest Producer of Antibiotics***

ANTIBIOTIC DIVISION, CHAS. PFIZER & CO., INC., BROOKLYN 6, N. Y.

Effective JUNE 1, 1952

DR. STANLEY E. BRADLEY

will become the EDITOR-IN-CHIEF of the

JOURNAL OF CLINICAL INVESTIGATION

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

On this date

The EDITORIAL AND BUSINESS OFFICES will move

from

JOURNAL OF CLINICAL INVESTIGATION
CINCINNATI GENERAL HOSPITAL
CINCINNATI 29, OHIO

to

JOURNAL OF CLINICAL INVESTIGATION
PRESBYTERIAN HOSPITAL
620 WEST 168TH STREET
NEW YORK 32, NEW YORK

THE *IN VITRO* METABOLISM OF ESTROGENS BY THE LIVER STUDIED BY MEANS OF A COLORIMETRIC METHOD[1,2]

By SEYMOUR LIEBERMAN,[3] HENRY J. TAGNON, AND PHYLLIS SCHULMAN

(From the Sloan-Kettering Institute for Cancer Research and Memorial Hospital, New York, N. Y.)

(Submitted for publication November 5, 1951; accepted January 21, 1952)

Since the work of Zondek (1) the *in vitro* action of liver slices and liver pulp on natural estrogens is usually called inactivation because the transformation undergone by these compounds results in loss of estrogenic activity as measured by bioassay methods (2). Using these methods, Heller and Heller (3) demonstrated that liver slices could inactivate estrone and estradiol *in vitro* and this work was confirmed by Singher and associates (4). Twombly and Taylor (5) showed that human liver inactivates 17-β estradiol less than does rat liver. It is generally accepted that liver mince is not as effective as slices in inactivating estradiol but De Meio, Rakoff, Cantarow and Paschkis (6), and Coppedge, Segaloff, Sarett and Altschul (7, 8) have shown that nicotinamide or diphosphorpyridine nucleotide enhance the activity of liver mince.

Thus the demonstration that the liver possesses the ability to inactivate estrogens has been based on bioassay methods. Until now colorimetric methods for estrogens have not been found satisfactory for the measurement of small amounts of estrogens in the presence of tissue (9) and therefore have not been suitable for the study of the metabolism. In this paper we are reporting a procedure which permits the simple colorimetric estimation of microgram amounts of estrogens, recovered after *in vitro* metabolic experiments with tissues. One of the advantages of this procedure is that it facilitates the study of the quantitative aspects of the reaction. This paper also presents the results obtained by this colorimetric technique in a study of the metabolic action of rat liver on 17-β estradiol, estrone and estriol. A subsequent paper will present the results obtained with human liver and will discuss their pertinence to the problem of hyperestrinism in humans.

METHODS

1) Colorimetric method: The procedure used in this work is based on the measurement of the yellow color produced when the phenolic estrogens are coupled with tetrazotized dianisidine. This reaction was first proposed by Talbot and associates (10) and subsequently modified by Bender and Wilson (11) for the assay of estrogens in the urine. The pure estrogens,[4] 17-β estradiol, estrone or estriol, were dissolved in 0.3 cc. of 95% redistilled ethyl alcohol. One tenth cc. of cold tetrazotized dianisidine solution was added, followed immediately by 0.75 cc. of cold 2% sodium carbonate solution. After mixing by swirling, 8 cc. of 20% sodium chloride and 20 cc. of toluene were added to the mixture. All these operations were carried out in an ice bath.[5] The colored material was extracted into toluene by shaking for 10 minutes in a shaking machine. After centrifuging for two minutes at 2,500 r.p.m., the toluene layer was pipetted off and read in a Beckman quartz spectrophotometer (Model DU) at 425 mμ in a 1 cm. cell against the blank. The blank was treated as the experimental sample except that no estrogen was present.

Quantitative measurements of 17-β estradiol, estrone and estriol were obtained by this procedure between 10 and 150 micrograms, as indicated on Figure 1. The relation between optical densities and concentrations followed Beer's law between these two values. The slope of the curve was found to be similar for estrone and estradiol but different for estriol (Figure 1).

The absorption spectrum of the colored compound showed a plateau-like peak between 400 mμ and 450 mμ with the maximum at 425 mμ approximately (Figure 2).

[1] Presented in part at the annual meeting of the American Society for Clinical Investigation, April 30, 1951, Atlantic City, N. J.

[2] This work was supported in part by a grant from the National Cancer Institute of the U. S. Public Health Service.

[3] Present address: Columbia University, College of Physicians and Surgeons, New York, N. Y.

[4] Kindly supplied by Drs. E. Schwenk and E. B. Hirshberg, Schering Corporation, Bloomfield, New Jersey.

[5] The tetrazotized dianisidine solution was prepared as follows: 25 mg. of dianisidine dihydrochloride (recrystallized from ethanol) were dissolved in 3 cc. of water. Three tenths cc. of concentrated hydrochloric acid, followed by 0.6 cc. of freshly prepared 5% sodium nitrite solution were added. After thorough mixing and a five minute waiting period, 0.60 cc. of a 5% urea solution was added. The solution was prepared in an ice bath, and made freshly for each experiment.

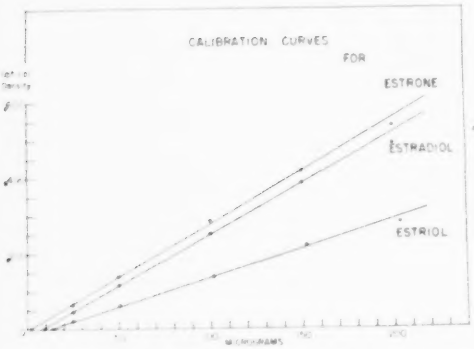


FIG. 1. CALIBRATION CURVES FOR ESTROGENS TREATED WITH TETRAZOTIZED DIANISIDINE

2) Incubation experiments: Normal albino rats, kept on a diet of purina laboratory chow, supplemented with rolled oats and flower seeds, were killed by a blow on the head, the liver obtained and sliced immediately. Three hundred mg. of liver slices (approximately 0.5 mg. thick) were incubated with continuous shaking in an open vessel with 100 micrograms of estradiol, estrone or estriol dissolved in 0.2 cc. of 95% redistilled ethanol. Incubation was carried out in 6 cc. of Krebs solution, pH 7.4 at 37.5° C. As a control, 300 mg. of the same liver were boiled for 30 minutes and similarly incubated with the same amount of estrogen. As a blank, 300 mg. of fresh liver slices were also incubated without the addition of estrogen. At the end of the period of incubation the reaction was stopped by immersing the flasks in a boiling water bath for 30 minutes.

Thereafter, each sample was submitted to alkaline hydrolysis by adding enough solid barium hydroxide (3.8 gms. of $Ba(OH)_2 \cdot 8\ H_2O$) to make a 4 N solution and boiling in an oil bath for 17 hours. At the end of the period of hydrolysis the samples were allowed to cool and were brought to approximately pH 2 by the addition of 7 cc. 4 N hydrochloric acid. The acid solution was then continuously extracted for 17 hours with redistilled benzene (approximately 60 cc.). The residue left after the evaporation of the benzene was dissolved in 0.3 cc. of 95% redistilled ethanol and assayed colorimetrically by the method described above. The value of the control was subtracted from the value of the experimental sample.

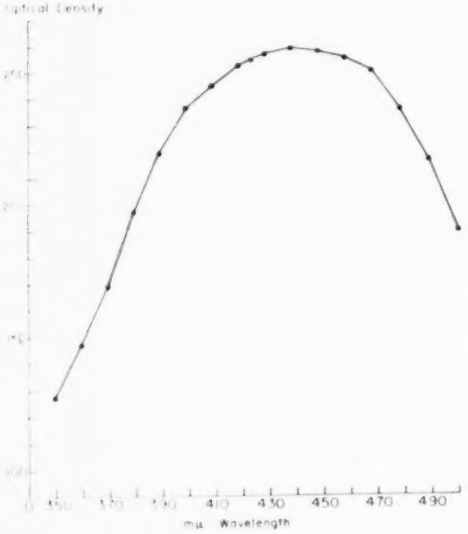


FIG. 2. ABSORPTION SPECTRUM OF THE COLORED COMPOUND OBTAINED BY TREATMENT OF 17-β ESTRADIOL, ESTRONE OR ESTRIOL BY TETRAZOTIZED DIANISIDINE

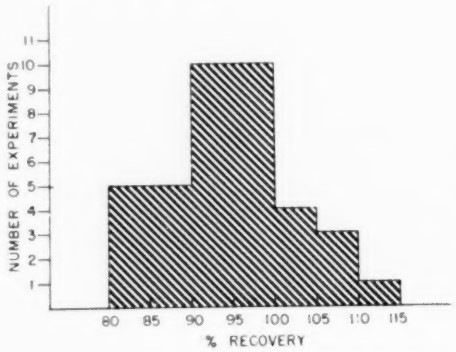


FIG. 3. RECOVERY EXPERIMENTS WITH 17-β ESTRADIOL

3) Recovery experiments: One hundred micrograms of 17-β estradiol were incubated for three hours with boiled rat liver slices. Recoveries ranged from 80% to 114% in 38 experiments with 20 experiments showing recoveries between 90 and 99% (Figure 3). The mean recovery value of these 38 experiments was 94% and the mean deviation or sigma value was 7%. In five experiments with estrone and six experiments with estriol the average recovery was 81% and 73%, respectively, with an average deviation from mean of 4% and 9% respectively (Table I).

TABLE I

Recovery of 100 micrograms of estrogen incubated with boiled rat liver

Estrogen	No. of exps.	Mean recovery (micrograms)	Average dev. from mean (micrograms)
β Estradiol	38	94	7
Estrone	5	81	4
Estriol	6	73	9

The use of barium hydroxide hydrolysis improved the recoveries. This was shown by a series of experiments designed to determine the effect, on the recovery of estrogens, of barium hydroxide hydrolysis of the liver tissue prior to extraction. Boiled rat liver slices were incubated with 100 micrograms of estradiol for 45 minutes at 37.5° C. in 6 cc. of Krebs solution. Thereafter, paired samples were extracted with and without prior hydrolysis. In 16 such experiments the average recovery of barium hydroxide hydrolyzed samples was 94 micrograms with a sigma value of 10%, and that of non-hydrolyzed samples was 71 micrograms with a sigma value of 17%.

Good recoveries were obtained with a 17 hour hydrolysis. Hydrolysis by means of sodium hydroxide did not yield as satisfactory results as those with barium hydroxide. As shown by Warner (12) barium hydroxide effects a more complete hydrolysis of proteins than sodium hydroxide, and this could explain the better recoveries. Recoveries were not improved by treatment of the material with a number of reagents used in an attempt to dissociate a hypothetical adsorption complex between liver tissue and estrogen. Specifically, the following reagents were ineffective: barium nitrate, urea and ammonium sulfate.

Hydrolysis with barium hydroxide for less than five hours gave incomplete recoveries, but when carried out overnight (approximately 17 hours) satisfactory results were obtained. Control experiments showed that such hydrolysis did not result in any destruction of the estrogens.

4) Extraction with benzene: Although continuous benzene extraction of a solution of pure 17-β estradiol in 6 cc. of Krebs solution resulted in complete extraction in a period of $1\frac{1}{2}$ hours, it was found that the length of time necessary to extract the estrogen in the presence of liver hydrolysate had to be increased to 17 hours in order to be optimally effective.

5) Color reaction: It was found necessary to carry out the color reaction on the benzene extract immediately, because the dried benzene extract could not be kept at room or even at refrigerator temperature for 24 hours without considerable loss of color-producing material. The nature of the reaction leading to this loss in the dried extract has not been investigated. However, it was found that the extracts could be kept for at least three days in the deep freeze (− 15° C.) without loss of color-producing material.

RESULTS

The results of metabolic experiments were obtained by subtracting the amount of estrogen recovered at the end of the incubation period from the mean value of estrogen obtained in the recovery experiments (Methods: section *3*).

1) Metabolism of 17-β estradiol, estrone or estriol by liver slices as measured colorimetrically: When 100 micrograms of estradiol were incubated at 37° C. with 300 mg. of fresh liver slices for three hours, the decrease in color-producing material expressed as estradiol, averaged 50 micrograms in 19 experiments using eight different rats (Table II). The amounts of estradiol metabolized by different liver samples ranged from 22 to 70 micrograms.

TABLE II

Amount of estrogens metabolized by fresh rat liver
(pH, 7.4; Temp., 37.5° C.; Amount of liver, 300 mg.)

Estrogen	No. of exps.	Avg. amt. metabolized (*micrograms*)	Avg. deviation from mean (*micrograms*)
17-β estradiol	19	50	13
Estrone	6	53	4
Estriol	6	21	11

The results of similar experiments using estrone or estriol as substrates are also shown in Table II. In six experiments using 100 micrograms of estrone, the average amount metabolized was 53 micrograms, with a deviation from the mean of 4 micrograms. When 100 micrograms of estriol were used as a substrate, the average amount metabolized was 21 micrograms with a deviation from the mean of 11 micrograms.

2) Effect of acid hydrolysis: In an attempt to determine whether the disappearance of estrogens was due to conversion to a benzene-insoluble conjugate, one experiment was carried out using acid hydrolysis (to cleave sulfates or glycuronidates) following the usual barium hydroxide hydrolysis of the proteins. Acid hydrolysis was carried out by adding 5 cc. of concentrated hydrochloric acid to the alkaline hydrolysate and heating at 100° C. for one hour. The results show (Table III) that this procedure did not increase the amount of estrogen recovered.

TABLE III

Recovery after incubation of 100 micrograms of 17-β estradiol with 300 mg. rat liver—with and without acid hydrolysis

Type of exp.	No. of exps.	Recovery (*per cent*)	
		Without acid hydrolysis	With acid hydrolysis
Incubation with fresh rat liver	3	52	56
Incubation with boiled liver (control)	1	85	83

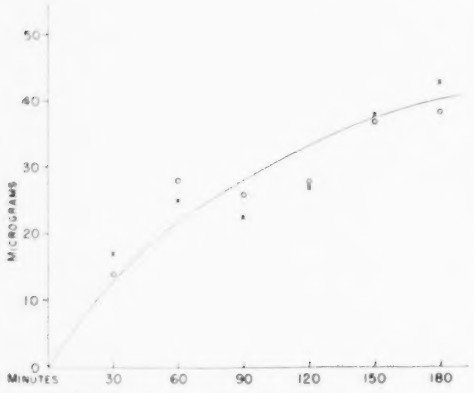


FIG. 4. METABOLISM OF 17-β ESTRADIOL BY RAT LIVER SLICES—EFFECT OF TIME OF INCUBATION

3) Effect of time and pH: Three hundred mg. portions of the same rat liver were incubated with 100 micrograms of estradiol for 30, 60, 90, 120 and 180 minutes, respectively. The amounts metabolized after each time interval (Figure 4) showed a steady increase over a period of three hours.

The optimum pH of the reaction was found to be between pH 6 and 7 as shown in Figure 5.

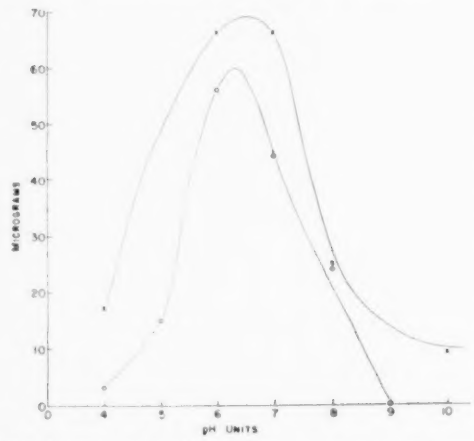


FIG. 5. EFFECT OF pH ON METABOLISM OF RAT LIVER SLICES ON 17-β ESTRADIOL

DISCUSSION

An accurate quantitative evaluation of the *in vitro* metabolic action of liver on estrogens is difficult by the bioassay method because each determination involves a great number of injections in large series of assay animals. The limitations of the bioassay made it desirable to develop a colorimetric method capable of measuring estrogens in metabolic experiments *in vitro*. The method presented here satisfies this requirement. Its accuracy and simplicity make it possible to do a great number of quantitative experiments with much less expenditure of time than with bioassay. This method, obviously, does not measure the metabolic action of tissue in terms of loss of estrogenic activity, but rather measures a chemical change in the molecule of the substrate resulting in a loss of color. This change consists of an unknown alteration of the estrogen molecule which makes it unavailable for extraction or estimation by this method. Therefore, no correlation need necessarily exist between the results obtained by this chemical method, and the bioassays in biological experiments. For instance, estrone and 17-β estradiol in equal amounts have widely different estrogenic activities in most test animals (13), while by this colorimetric method they give approximately the same color intensity. A quantitative metabolic conversion of estradiol into estrone would not be measured by our method, but would be detected by the bioassay.

There are several aspects of the method described here which deserve comment: liver and other tissues on continuous extraction with benzene yield a certain amount of color-producing material. Therefore, it is necessary to run a liver blank with the actual experimental sample to make proper allowance for the non-specific color. The duration of extraction advocated here has been found to be adequate for complete extraction of the estrogens without extraction of excessive amounts of extraneous material. The experimental data presented here demonstrate the necessity of completely hydrolyzing the tissue prior to extraction in order to achieve complete recoveries. This fact suggests that a portion of the estrogenic compounds becomes bound to the liver tissue during the incubation period in such a way that barium hydroxide hydrolysis alone, of all the procedures tested by us, could make it available for extraction. The nature of this binding between tissue and estrogens has not been studied here. In this respect, it should be mentioned that Lehninger and Scott (14) have re-

ported a similar binding of estradiol by prostate tissue *in vitro*. They did not test the action of barium hydroxide hydrolysis on this binding.

Our results show that the rat liver is able to metabolize larger amounts of estradiol *in vitro* than previous experimental work would lead us to believe, namely, an average amount of 50 micrograms for 300 mg. of liver in three hours at 37° C. The data indicate also that the reaction proceeds at a rather constant rate for at least three hours; this rate constitutes, therefore, a valid one for comparison of activity under different experimental conditions. The nature of the end products of the reaction between liver and estrogens *in vitro* is being studied and will be reported later. However, the data show that 17-β estradiol, estrone and estriol are all metabolized, as measured by the colorimetric method. This indicates that the products of the action of liver slices on estradiol probably do not consist solely of estrone and estriol. A conversion of estradiol into estrone would not account for the results reported here while a conversion into estriol could do so. The possibility and extent of such a conversion is being investigated now.

The data show also that acid hydrolysis of the barium hydrolysate did not increase the amount of color-producing material recovered in the benzene extract, indicating that the action of liver is not a conjugation. This constitutes a confirmation of the findings of Heller and Heller (3).

SUMMARY

1) A method based on colorimetric assay is described for the study of the metabolic action of liver and other tissue on natural estrogen. The method permits measurement of 10 to 150 micrograms of 17-β estradiol, estrone and estriol with the sigma value of 7%, in the presence of tissue.

2) By this method it was found that 300 mg. of rat liver slices metabolize on the average 50 micrograms of estradiol, 53 micrograms of estrone, and 21 micrograms of estriol in three hours at 37.5° C. and pH 7.4.

3) Evidence was presented that estradiol becomes bound to liver tissue in such a way that complete hydrolysis is necessary before it can be extracted.

4) The end products of the reaction of liver on 17-β estradiol are unknown but the data suggest that they do not consist solely of estriol, estrone or conjugated estrogens.

REFERENCES

1. Zondek, B., Über das Schicksal des Fallikelhormons (Follikulin) im Organismus. Skandinav. Arch. f. Physiol., 1934, **70**, 133.
2. Jailer, J. W., The metabolism of the estrogens: a review. J. Clin. Endocrinol., 1949, **9**, 557.
3. Heller, C. J., and Heller, E. J., Metabolism of estrogens: Effect of pregnancy upon metabolism *in vitro* of estrone, estradiol and estriol. Endocrinology, 1943, **32**, 64.
4. Singher, H. O., Kensler, C. J., Taylor, H. C., Rhoads, C. P., and Uma, K., The effect of vitamin deficiency on estradiol inactivation by liver. J. Biol. Chem., 1944, **154**, 79.
5. Twombly, G. H., and Taylor, H. C., Jr., Inactivation and conversion of estrogens *in vitro* by liver and other tissues from human cancer patients and from mice of strains susceptible to mammary carcinoma. Cancer Research, 1942, **2**, 811.
6. De Meio, R. H., Rakoff, A. E., Cantarow, A., and Paschkis, K. E., Mechanism of inactivation of α-estradiol by rat liver "in vitro." Endocrinology, 1948, **43**, 97.
7. Coppedge, R. L., Segaloff, A., Sarett, H. P., and Altschul, A. M., Cozymase as a part of the hepatic estrogen-inactivating system. J. Biol. Chem., 1948, **173**, 431.
8. Coppedge, R. L., Segaloff, A., Sarett, H., and Altschul, A., Cozymase in the hepatic inactivation of α-estradiol. J. Clin. Endocrinol., 1948, **8**, 602.
9. Haslewood, G. A. D., *in* Hormone Assay, edited by Emmens, C. W. Academic Press, Inc., New York, 1950, pp. 456–458.
10. Talbot, N. B., Wolfe, J. K., MacLachlan, E. A., Karush, F., and Butler, A. M., The colorimetric assay of weakly phenolic ketones, "estrone," in extracts of human urine. J. Biol. Chem., 1940, **134**, 319.
11. Bender, A. E., and Wilson, A., An investigation into the chemical estimation of the estrogens in urine. Biochem. J., 1947, **41**, 423.
12. Warner, R. C., The alkaline hydrolysis of egg albumin. J. Biol. Chem., 1942, **142**, 741.
13. Pincus, G., *in* The Hormones: Physiology, Chemistry and Application, edited by Pincus, G., and Thimann, K. V. Academic Press, Inc., New York, 1948, pp. 341–343.
14. Lehninger, A. L., and Scott, W. W., The action of human prostate slices on α-estradiol and estrone. Endocrinology, 1947, **40**, 9.

THE METABOLISM OF ESTROGENS BY THE HUMAN LIVER STUDIED *IN VITRO*[1,2]

By HENRY J. TAGNON, S. LIEBERMAN,[3] PHYLLIS SCHULMAN, AND ALEXANDER BRUNSCHWIG

(From the Sloan-Kettering Institute for Cancer Research and Memorial Hospital, New York, N. Y.)

(Submitted for publication November 5, 1951; accepted January 21, 1952)

In the preceding paper (1) a method was described which permits the simple colorimetric estimation of microgram amounts of estrogens in the presence of tissues. The results obtained by the use of this method in a study of the *in vitro* metabolic action of rat liver on 17-β estradiol were also reported.

In the present paper we are reporting a similar study on human liver. The original motive for this study was to evaluate whether a deficiency of the metabolic function of the liver exists in patients with cancer of the uterus. Patients with this type of cancer and other diseases were studied.

METHODS

The procedures described in the preceding paper for the incubation of liver slices, the extraction of the estrogens and the colorimetric method for the measurement of estrogens were used in the present work and found to be applicable without modification to the study of human liver.

Human liver specimens were obtained at laparotomy from patients with various surgical diseases. The patients were unselected. The liver specimen was immersed into ice-cold Krebs solution in the operating room, immediately transferred to the laboratory and the incubation experiment begun. The time interval between surgical removal of the specimen and the beginning of the incubation period did not exceed 10 minutes and was comparable to the time interval obtaining in the experiments with rat liver.

The liver specimen consisted of a wedge of tissue resected from the anterior border and weighing approximately 2 gms. Sufficient tissue was available in each case to run the metabolic, the control and the blank experiments with slices from the same bloc of tissue.

After slices were obtained from the liver biopsy, the remaining tissue was fixed in formalin and sectioned for morphologic examination.[4] Thus the histologic sections in each case came from the area immediately adjacent to that furnishing the slices for the metabolic experiments.

There were three male and nine female patients in this series ranging in age from 31 to 75. All were white except Case 3 who was a Negro.

The overall clinical and laboratory data pertinent to this study are presented in Table I. The diagnoses were based on surgical exploration and pathologic examination of operative specimens. All patients received preoperative medication in the form of the usual dose of atropin, nembutal and morphine or demerol. All patients received a general anesthesia by ether and oxygen. Liver biopsies were usually obtained shortly after laparotomy. Additional data of significance for certain patients are presented as follows.

Case 1: This patient had a carcinoma of the cervix for which she underwent a radical hysterectomy in December, 1947. Recurrent carcinoma was discovered in December, 1950 and she then underwent the operation during which a liver biopsy was obtained.

Case 2: This patient had an epidermoid cancer of the cervix, leiomyoma, atrophic endometrium and chronic salpingitis. A radical hysterectomy was performed in March, 1951 and she underwent a laparotomy for recurrence of the cancer in July, 1951, at which time the liver biopsy was obtained.

Case 5: This patient had an epidermoid cancer of the cervix treated eighteen years ago by radium and X-ray. On February 20, 1951, an exploratory laparotomy was performed and multiple peritoneal metastatic implants were discovered. There were no liver metastases.

Case 6: This patient had a papillary adenocarcinoma of the cervix for which she underwent a total hysterectomy and bilateral ovariectomy on June 30, 1950. X-ray therapy was given from September 11, 1950 to October 9, 1950. On March 22, 1951 she had an exploratory laparotomy which failed to demonstrate the presence of cancer. The liver biopsy was obtained during this laparotomy.

Case 8: This patient underwent a left radical mastectomy for breast carcinoma in 1948. On March 30, 1951 she underwent a partial hepatectomy (left lobe and anterior portion of the right lobe immediately anterior to the porta hepatica) for liver metastasis. The metabolic ex-

[1] Presented in part at the 1951 meeting of the American Society for Clinical Investigation, April 30th, Atlantic City, N. J.

[2] This work was supported in part by a grant from the National Cancer Institute of the U. S. Public Health Service.

[3] Present address: Columbia University, College of Physicians and Surgeons, New York, N. Y.

[4] Microscopic examination of the liver specimens was performed by Dr. Arthur Allen of the Department of Pathology.

TABLE I

Metabolism of estradiol by human liver in vitro

300 mg. of liver incubated with 100 micrograms of 17-β estradiol for three hours at 37° C.

Case No.	Age	Diagnosis	Operation	Recovery with boiled liver	Recovery with fresh liver	Amount metabolized
				micrograms	*micrograms*	*micrograms*
1 CC	68	Recurrent carcinoma cervix	Excision	94	51	43
2 WM	53	Epidermoid carcinoma cervix	Excision	94	65	29
3 FL	31	Epidermoid carcinoma cervix	Hysterectomy	83	57	26
4 AM	39	Adenocarcinoma corpus uteri	Hysterectomy	107	62	45
5 HA	75	Papillary adenocarcinoma uterus	Exploratory laporatomy	65	42	23
6 TM	30	No disease	Exploratory laporatomy	76	73	3
7 ST	30	Ovarian cyst benign fibromyoma	Removal of cyst	81	59	22
8 ER	44	Metastatic mammary carcinoma to liver	Partial hepatectomy	87	57	30
9 AG	41	Cancer breast—hemochromatosis	Bilateral oophorectomy	95	54	41
10 GC	63	Adenocarcinoma colon	Colectomy	90	59	31
11 SM	53	Metastatic carcinoma liver	Partial hepatectomy	90	69	21
12 VR	50	Metastatic carcinoma liver—biliary cirrhosis	Exploratory laporotomy	99	62	37

Summary of results

I. Boiled liver incubated with 100 micrograms 17-β estradiol

No. of experiments	12
Mean recovery	88
Mean deviation	11

II. Fresh liver incubated with 100 micrograms 17-β estradiol

No. of experiments	12
Mean recovery	59
Mean deviation	8
Mean amount metabolized	29 micrograms
Mean deviation	11

periment in this case was carried out with liver tissue free of metastatic contamination.

Case 9:[5] This patient had a left radical mastectomy two years previously for mammary carcinoma. In May, 1951 she presented evidence of mammary carcinoma of the right breast and of hemochromatosis (diagnosis based on a biopsy of the skin and of the liver). Mild diabetes was present. Serum bilirubin level was 0.6 mg. Bromsulfalein retention at 45 minutes was 9%, cephalin flocculation test was 1 plus at 48 hours, thymol turbidity was 6.7 cc. of barium sulfate suspension (*2*); alkaline phosphatase was 3.7 glycerophosphate units. On May 14, 1951 she underwent a laparotomy for bilateral oophorectomy, during which the liver biopsy was obtained. A photograph of a section of the liver is shown in Figures 1 and *2*; it reveals marked alteration in lobular architecture with striking periportal fibrosis and a conspicuous increase in lymphocytes and histiocytes in the periportal areas. In addition there is an abundant hemosiderosis not only of the periportal histiocytes but also of the epithelium of bile ducts and of the parenchymal cells. No Mallory bodies were present. Stains for hemofuscin were not done. A skin biopsy was also examined and diagnosed as hemochromatosis.

Case 11: This patient had an abdomino-perineal resection for carcinoma of the rectum six years previously and

[5] We are indebted to Dr. Guy Robbins for biopsy specimens and permission to use this case.

FIG. 1. PHOTOMICROGRAPH OF SECTION OF LIVER FROM CASE 9 SHOWING THE HEMOSIDEROSIS, FIBROSIS AND LOBULAR IRREGULARITY OF HEMOCHROMATOSIS

Magnification 60 ×. Hematoxylin eosin.

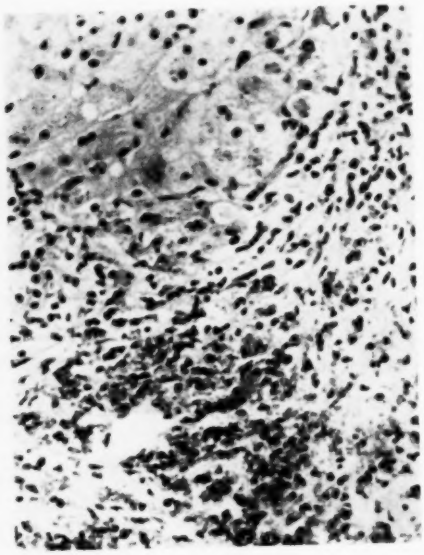


FIG. 2

Same as Figure 1. Magnification 400 ×.

underwent a left partial hepatectomy for a single metastatic nodule 10 cm. in diameter located in the left lobe of the liver. Liver tissue for the metabolic experiment was obtained from the tumor-free portion of the surgical specimen; morphological examination showed the experimental portion of tissue to be entirely normal. There was no clinical or laboratory evidence of liver dysfunction in this patient.

Case 12: This patient underwent an exploratory laparotomy for jaundice which revealed multiple metastases in both lobes of the liver; the primary tumor was unknown. A biopsy from a region of the liver adjacent to the metastatic tumor was used for the metabolic experiment; morphologic examination of this biopsy showed biliary cirrhosis with acute and subacute cholangiolitis (Figure 3). There was also a very small subcapsular focus of metastatic adenocarcinoma seen in this section.

The serum bilirubin level in this patient was 6.6 mg. but no other evidence of liver dysfunction was present.

In summary, there were two patients in this series (Nos. 9 and 12) who showed clinical and pathologic evidence of liver diseases. In addition two patients (Nos. 8 and 11) had metastatic liver disease but showed no clinical evidence of liver disease and the biopsy specimens used for metabolic experiments showed an entirely normal structure. The remaining eight patients had normal livers by clinical observation and morphologic examination.

RESULTS

1) Recovery experiments: In the previous paper (1) recovery experiments were carried out with boiled rat liver slices in order to test the accuracy of this method. Similar experiments with boiled human liver slices were carried out here. In 12 experiments using one hundred micrograms of 17-β estradiol incubated with 300 mg. of boiled human liver slices for three hours, the mean recovery was 88 micrograms with a mean deviation or sigma value of 11 micrograms.

2) Metabolism of estradiol 17-β by human liver slices: When 100 micrograms of estradiol were incubated at 37° C. with 300 mg. of fresh liver slices for three hours, the decrease in color-producing material expressed as estradiol averaged 20 micrograms in 12 experiments using specimens from 12 different patients; the sigma value was 8 micrograms.

Individual experiments are shown in Table I. In each case a recovery and a metabolic experiment were carried out simultaneously with slices from the same bloc of tissue. The amount of 17-β estradiol metabolized was calculated by subtracting

FIG. 3. PHOTOMICROGRAPH OF SECTION OF LIVER FROM CASE 12 SHOWING THE PROLIFERATION OF BILE DUCTS AND PERIPORTAL FIBROSIS.

Magnification 200 ×. Hematoxylin eosin.

the amount recovered after incubation of fresh tissue with estradiol from that obtained after incubation of boiled tissue with estradiol. The results show great individual variations extending from 3 micrograms to 45 micrograms. No correlation could be found in this series between the metabolic activity and the nature of the disease, the age or other data from the clinical history. Of special interest is the fact that the liver from the three patients with cancer of the cervix uteri (Nos. 1, 2 and 3) and the one patient with cancer of the corpus uteri showed an *in vitro* metabolic activity well within the average values for the whole series. Also, it is noteworthy that the livers of the two patients (Nos. 9 and 12) with definite clinical and pathologic evidence of serious liver disease did not show an impaired *in vitro* metabolic activity.

DISCUSSION

The 12 patients studied here represent a heterogeneous group of diseases. Metabolic experiments of the type carried out here could not be done on entirely normal subjects because laparotomy was required to obtain the large liver biopsies which were needed. Since none of the subjects was entirely normal, no absolute conclusion on the normal *in vitro* metabolic activity of human liver towards estradiol can be deduced from the data reported here. However, there are several factors which suggest that the average amount of estradiol found to be metabolized by human liver in our experiments may not be very different from what normal human liver can metabolize *in vitro*. These factors are: *1*) In this heterogeneous group of patients of different ages and with different types of tumors, the amount of estradiol metabolized was fairly uniform as shown by the sigma value of 11 micrograms for the whole group. *2*) Two liver specimens (from Cases 9 and 12), which showed pathologic evidence of advanced liver disease, had a metabolic *in vitro* activity well within the average range of values for this series. Since all other liver specimens were morphologically normal, it would appear that the metabolic function of liver studied here may be unimpaired even in the presence of structural changes indicative of serious liver damage. *3*) One patient in this group (Case 7) did not have cancer and the metabolic activity of her liver was not greater than that of the other patients who had cancer. *4*) The amount of estradiol found to be metabolized by this group of patients, although smaller than the average amount metabolized by rat liver *in vitro*, was of the same general order of magnitude. The rat is considered to be one of the most active species as far as this metabolic function is concerned and our results indicate that human liver differs only to a moderate degree from the rat liver.

On the basis of the results obtained here, one can conclude that an average human liver (weighing 15,000 gms.) is able to metabolize *in vitro* approximately 11.5 gms. of 17-β estradiol per 24 hours at 37° C. No data are available as yet to indicate in which way this very high level of "*in vitro*" activity is related to the physiologic activity "*in vivo*."

Although none of these arguments is decisive, taken together they suggest that our results give a valid indication of the capacity of the human liver to metabolize estradiol *in vitro*. However, the effect of the pre-operative medication and general anesthesia on this metabolic reaction can not be accurately evaluated. The data also show that this metabolic function was not impaired in three patients with cancer of the cervix and one patient with cancer of the fundus. This fact is interesting in view of the many attempts in recent years to attribute to hyperestrinism an etiological role in cancer of the uterus (3, 4). Certain authors have postulated on the basis of indirect evidence that impairment of the estrogenic metabolic function of the liver was associated with the presence of cancer of the cervix and the fundus (3, 4). Our data do not support this hypothesis but indicate that advanced disease of the liver as judged from clinical and morphologic observation may not affect its *in vitro* metabolic activity on estradiol. It seems that this metabolic function should not be impaired in mild liver disease and does not constitute a sensitive test of liver function.

Twombly and Taylor have shown that human liver is able to metabolize estradiol *in vitro* (5). Our data confirm their conclusion and give information, in addition, on the quantitative aspect of the reaction. However, it is well to remember that the method of study of the metabolic function of the liver used here does not necessarily measure the

same metabolic alteration which results in inactivation of the estrogens as measured by the bioassay. The possibility has not been ruled out in these experiments that the metabolites from estradiol metabolism by liver are different in patients with cancer of the uterus and other patients or normals. This possibility will be discussed in a future report.

REFERENCES

1. Lieberman, S., Tagnon, H. J., and Schulman, P., The *in vitro* metabolism of estrogens by the liver studied by means of a colorimetric method. J. Clin. Invest., 1952, **31**, 341.
2. Tagnon, H. J., and Trunnell, J. B., Studies of liver function in patients having cancer of the breast. Cancer, 1948, **1**, 472.
3. Ayer, J. E., and Bauld, W. A. G., Thiamine deficiency and high estrogen findings in uterine cancer and in menorrhagia. Science, 1946, **103**, 441.
4. Biskind, M. S., Nutritional therapy of endocrine disturbances. Vitamins & Hormones, 1946, **4**, 147.
5. Twombly, G. H., and Taylor, H. C., Jr., Inactivation and conversion of estrogens *in vitro* by liver and other tissues from human cancer patients and from mice of strains susceptible to mammary carcinoma. Cancer Research, 1942, **2**, 811.

COMPARATIVE EFFECTS OF A PURIFIED AND AN ADEQUATE DIET ON THE COURSE OF FATTY CIRRHOSIS IN THE ALCOHOLIC[1]

By GERALD B. PHILLIPS, GEORGE J. GABUZDA, JR.,[2] AND CHARLES S. DAVIDSON

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services [Harvard], Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Mass.)

(Submitted for publication November 5, 1951; accepted January 21, 1952)

The association of alcoholism and cirrhosis of the liver is widely recognized; nevertheless, it is still not clear whether the hepatic disturbance is the result of (*1*) an hepatotoxic effect of the alcohol or a contaminant it may contain, (*2*) a nutritional deficiency, or (*3*) a combination of the two. The concept that cirrhosis in the alcoholic may be a deficiency disease has gained popularity ever since the demonstration that fatty cirrhosis similar to that seen in man could be produced in animals by means of low protein diets (*1, 2*). In support of this view is the association of fatty cirrhosis with states of malnutrition unaccompanied by alcoholism (*3, 4*). Moreover, the apparent therapeutic efficacy of a nutritious diet, as reported by Patek and Post (*5, 6*), further suggests nutritional deficiency as an etiologic factor. But whether the success of this regimen resides in the diet, withdrawal of alcohol, bed rest, or in a combination of these factors, is as yet unsettled.

Recently, Eckhardt and his associates (*7*) followed three chronic alcoholics with fatty cirrhosis maintained for eight to 13 days on a diet essentially devoid of protein but adequate in calories and supplemented with a multivitamin preparation.[3] These patients showed striking improvement in clinical status and in hepatic function tests within a week, although histologically liver fat did not decrease significantly except in one patient during a period of choline supplementation.

To elucidate further the role of diet, three chronic alcoholics with large fatty livers were maintained on a highly purified diet. After eight to ten days of this regimen, an adequate diet was provided and the study continued for an additional eight to 19 days. Liver function, size, and histology were evaluated at appropriate intervals.

MATERIALS AND METHODS

Brief descriptions of the three patients included in this study follow.

Patient B. R. was a 56-year-old white male, chronic alcoholic who had been drinking heavily and eating meagerly for three weeks prior to admission. On entry, a firm tender liver edge was felt 4 cm. below the costal margin in the midclavicular line, but no edema, ascites, icterus, splenomegaly or spider telangiectasia was detected. Because he was thoroughly confused and manifested a marked tremor, severe ataxia, coarse nystagmus, and bilateral external rectus muscle paralysis, the diagnosis of Wernicke's syndrome with probable delirium tremens was made.

Patient P. F. was a 46-year-old white male who had been drinking steadily for 20 to 30 years and had had frequent episodes suggestive of delirium tremens. Nine months before the present admission, he had been hospitalized because of jaundice and was found to have hepatomegaly with marked functional impairment. Since that time he had been imbibing large quantities of alcoholic beverages and eating little. On admission, his liver edge was 10 cm. below the costal margin in the midclavicular line, firm, and non-tender. There was no evidence of edema, ascites, icterus, splenomegaly, or spider telangiectasia. He exhibited marked confusion, ataxia and bilateral external rectus weakness and was considered to have Wernicke's syndrome.

Patient R. W. was a 28-year-old white female who had been an alcoholic for about six years and had been drinking heavily and steadily for the last four to five years. She admitted to consuming only small quantities of food, often eating nothing for three to four days at a time. Icteric sclerae were noted several weeks prior to entry. No definite history of previous hepatic disturbance could be elicited. Physical examination on admission revealed scleral icterus, palmar erythema, numerous prominent

[1] The expenses of this investigation were defrayed in part by grants from Merck & Co., Inc., Rahway, New Jersey, and from the Commission on Liver Disease, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C., to Harvard University.

[2] Welch Fellow in Internal Medicine of the National Research Council.

[3] This diet maintained nitrogen balance and body weight in a normal adult male when casein was provided orally as the sole source of protein.

spider telangiectases, and a large tender liver, which extended almost to the iliac crest. Ascites and edema were not evident.

During the initial period of hospitalization these patients were given a purified diet which consisted of a solution containing 200 gm. of glucose and 1.3 gm. of sodium chloride per liter of water. B. R. and P. F. consumed approximately 2 liters of this solution daily (*i.e.*, about 1,600 calories and 2.6 gm. sodium chloride), while R. W., who frequently had nausea and vomiting following ingestion of the solution, was able to retain only about a liter (*i.e.*, about 800 calories and 1.3 gm. sodium chloride) a day. In addition, R. W. ingested 6 gm. of potassium chloride a day during the period of the purified diet. None of the patients received any sustenance after entering the hospital except for the glucose and minerals, which were begun within a few hours after admission and continued for eight days in the case of patients P. F. and R. W. and 10 days in the case of patient B. R. On cessation of the purified diet, an adequate diet was provided for the remainder of the patient's hospital stay. The adequate diet consisted of the unsupplemented regular hospital diet (2,000 to 2,200 calories—protein 80 to 100 gm., carbohydrate 200 to 250 gm., and fat approximately 110 gm. daily). An accurate measure was not made of the quantity of food ingested by each patient. B. R. and P. F. ate moderately well during the first week of the adequate diet, while R. W. consumed only a small fraction of what was offered to her, frequently refusing entire meals. After the first week of this diet, R. W.'s appetite and food intake gradually increased. No medication was administered after admission other than small doses of paraldehyde for B. R. and P. F. and penicillin during three days to P. F. because of cough without fever, increased chest markings by X-ray, and an elevated white blood count.

All patients were ambulatory after the second or third day in the hospital.

Because patients B. R. and P. F. had definite evidence of Wernicke's syndrome, thiamine was administered to both with subsequent improvement in their neurological abnormalities.*

Liver function, size, and histology were evaluated on the day of admission and at appropriate times during the study. To appraise hepatic function, the following measurements were made: bromsulphalein retention 45 minutes after the intravenous administration of the dye (5 mg. per Kg. of body weight calculated on the basis of initial body weight), "one minute" and total serum bilirubin (8), thymol turbidity and flocculation (9), urine urobilinogen (10) and bile (11), and plasma prothrombin concentration (12).

Punch biopsies of the liver were performed using the Vim-Silverman needle. In patient R. W. a subcostal anterior approach was employed, while in the other two patients, the needle was inserted transthoracically in the lower axilla. For serial biopsies, an attempt was made to insert the needle at the site of the previous puncture. However, a shift in the biopsy site may have taken place

TABLE I

Patient B. R., male, age 56

Day of study		0	5	8	10	18
Intake		Glucose and saline*				Adequate diet
Bromsulphalein, % retention		41	51	43	61	32
Serum bilirubin mg./100 cc.	"1 min."	0.92	0.76	0.63	0.31	0.27
	Total	2.3	1.8	1.7	1.2	0.74
Thymol turbidity† cc. $BaSO_4$			0.7	0.6	0.7	1.3
Thymol flocculation (0–4+)			0	0	0	0
Urine urobilinogen		1:256		1:256	1:256	1:64
Urine bile (0–4+)				2+	2+	0
Liver size‡		4 cm.		5 cm.		
Hepatic fat (0–4+)		4+		3+	4+	2+

* Patient received 100 mg. of thiamine hydrochloride subcutaneously twice a day from days 4 to 10, 1 gm. of niacin orally from days 7 to 10, and 4 cc. of an intravenous multivitamin preparation ("Berocca-C") on days 8 and 9.
† Normal value—<1.7 cc. $BaSO_4$ suspension.
‡ Below costal margin in midclavicular line.

TABLE II

Patient P. F., male, age 46

Day of study		0	6	8	16	22
Intake		Glucose and saline*			Adequate diet	
Bromsulphalein, % retention		37	40	45	31	26
Serum bilirubin mg./100 cc.	"1 min."	1.2	1.4	1.9	0.59	0.48
	Total	2.1	2.9	3.4	1.2	1.0
Thymol turbidity† cc. $BaSO_4$		4.1	3.6	4.9	3.3	3.6
Thymol flocculation (0–4+)		1+	1+	1+	1+	2+
Urine urobilinogen		1:256	1:512	1:512	1:256	1:128
Urine bile (0–4+)		2+	2+	2+	2+	1+
Liver size‡		10 cm.		10 cm.	9 cm.	
Hepatic fat (0–4+)		4+		4+	3+	2+

* Patient received 5 mg. thiamine hydrochloride intravenously on day 2, 15 mg. orally from days 3 to 6, and 50 mg. subcutaneously on days 7 and 8.
† Normal value—<1.7 cc. $BaSO_4$ suspension.
‡ Below costal margin in midclavicular line.

* B. R. received 100 mg. of thiamine hydrochloride subcutaneously twice a day from days 4 to 10, while P. F. was given 5 mg. intravenously on day 2, 15 mg. orally from days 3 to 6, and 50 mg. subcutaneously on days 7 and 8. In addition, B. R. received 1 gm. of niacin orally from days 7 to 10 and 4 cc. of an intravenous multi-vitamin preparation, "Berocca-C" (Hoffmann-LaRoche), on days 8 and 9. Each 2 cc. of this contains thiamine hydrochloride, 10 mg.; riboflavin, 6 mg.; niacinamide, 80 mg.; pyridoxine hydrochloride, 6 mg.; panthenol (equivalent to 6 mg. Ca pantothenate), 6 mg.; ascorbic acid, 100 mg.; with 3% gentisic acid ethanolamide as a preservative.

TABLE III

Patient R. W., female, age 28

Day of study		0	4	8	15	18	20	27
Intake		Glucose and saline*			Adequate diet			
Bromsulphalein, % retention		33	35	39	30	31	31	27
Serum bilirubin mg. *100 cc.*	"1 min."	1.2	1.1	1.0	0.52	0.48	0.56	0.48
	Total	2.5	2.5	2.2	1.3	1.0	1.0	0.99
Thymol turbidity† *cc. $BaSO_4$*		4.0	3.9	3.9	2.7	3.1	3.1	3.7
Thymol flocculation (0–4+)		3+	3+	3+	1+	1+	1+	1+
Urine urobilinogen		1:32	1:32	1:32	1:32	1:32		1:32
Urine bile (0–4+)		0	0	0	0	0		
Liver size, *cm.*‡		15	15	15	13	13		9
Hepatic fat (0–4+)		2+		2+		0		

* Patient received 6 gm. of potassium chloride daily from days 0 to 8.
† Normal value—<1.7 cc. $BaSO_4$ suspension.
‡ Below costal margin in midclavicular line.

due to changes in liver size, position of the patient, or phase of respiration. No untoward reactions occurred except in R. W., who developed a small tender mass, probably a hematoma, on the anterior surface of the liver after the first biopsy and experienced pain for one to two days after each subsequent one. The liver tissue was fixed in 10 per cent formalin, sectioned, and stained with hematoxylin and eosin. No other stains were used. In addition to the usual histological examination, fat, identified as large unstained vacuoles, was estimated on a 1+ to 4+ scale.[5]

RESULTS

The effects of the purified and adequate diets on hepatic function, size, and fat content are summarized in Tables I–III. The results obtained in the three patients studied are similar. During the purified diet period there was no evidence of hepatic functional improvement except for a diminution in serum bilirubin in two patients (B. R. and R. W.). The serum bilirubin increased in the third patient (P. F.). The impaired ability to excrete bromsulphalein exhibited by all three patients on admission was augmented still further during the period of the purified diet. Thymol turbidity and urine urobilinogen and bile either remained the same or became more abnormal. In no patient was there any detectable decrease in hepatic size.

An estimation of fat content and of the extent of fibrosis was made by microscopic examination of the stained sections of the liver biopsies (Figures 1–3). This method is at most semi-quantitative. In addition, the possibility exists that there is variation in these elements in different parts of the liver. B. R.'s initial liver biopsy showed marked fatty infiltration and minimal, if any, fibrosis (Figure 1). P. F., likewise, had a large amount of hepatic fat and in this instance definite fibrosis (Figure 2). R. W. had a moderate amount of hepatic fat but a considerable degree of fibrosis (Figure 3). The biopsies secured after eight days of the purified diet showed a slight decrease in hepatic fat in B. R. but no significant change in P. F. or R. W. A repeat biopsy performed on B. R. after ten days of the purified diet, however, disclosed a quantity of hepatic fat similar to that seen in the initial biopsy. Thus, a significant decrease in hepatic fat was not evidenced by any of the patients at the conclusion of the period of the purified diet.

Following the purified diet, the patients were given the adequate diet for the remainder of their hospitalization. Hepatic function was evaluated

[5] The authors are grateful to Dr. Frederic Parker, the Mallory Institute of Pathology, Boston City Hospital, for reviewing the histological sections and to Miss Lillian Leavitt of the same institution for preparing the biopsy specimens.

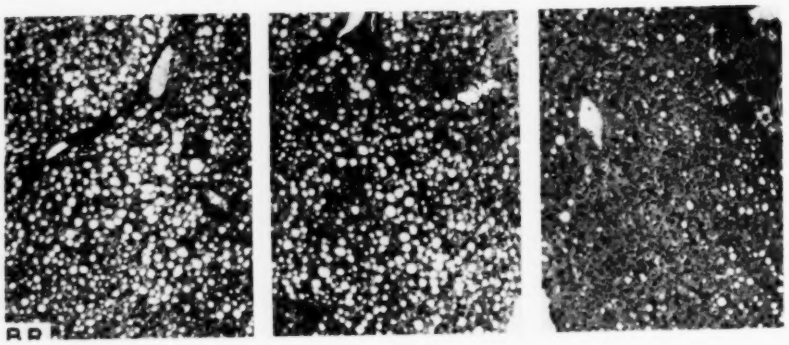


FIG. 1. PATIENT B. R.

The biopsies are in chronological sequence from left to right. The first biopsy was performed on admission, the second after ten days of the purified diet, and the third after eight days of the adequate diet. Hematoxylin and eosin × 50.

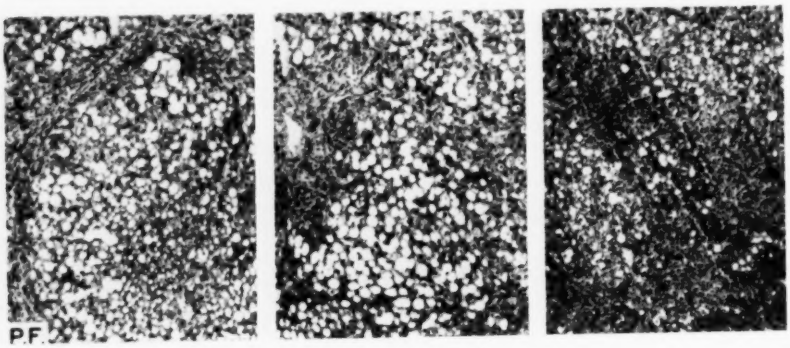


FIG. 2. PATIENT P. F.

The biopsies are in chronological sequence from left to right. The first biopsy was performed on admission, the second after eight days of the purified diet, and the third after 14 days of the adequate diet. Hematoxylin and eosin × 50.

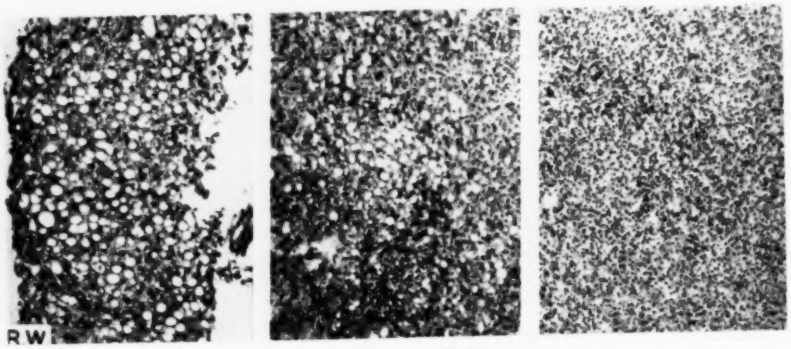


FIG. 3. PATIENT R. W.

The biopsies are in chronological sequence from left to right. The first biopsy was performed on admission, the second after eight days of the purified diet and the third after ten days of the adequate diet. Hematoxylin and eosin × 50.

after eight days of this diet in B. R. and P. F., and after seven days in R. W. Considerable improvement was apparent by the end of this period in all three patients. Both the B. S. P. retention and serum bilirubin concentration decreased significantly, and the urine urobilinogens which were abnormal also returned toward normal.

Liver size decreased definitely in R. W. and possibly in P. F. It was not remeasured in B. R. There was a marked diminution in hepatic fat in B. R. and R. W. after eight and ten days, respectively, of the adequate diet, but the decrease was slight, if any, in P. F. after eight days. A repeat biopsy in P. F. after 14 days of the adequate diet, however, revealed a definite decrease in hepatic fat.

DISCUSSION

That most chronic alcoholics with fatty cirrhosis improve rapidly when hospitalized and provided with a regular hospital diet has been the experience of this clinic as well as of others (13–17). But the relative importance of food, alcohol withdrawal and bed rest in this rapid recovery has not been clarified sufficiently. Although fatty cirrhosis can be produced in animals by means of low protein diets and prevented by methionine or choline, Eckhardt and his coworkers (7), in a previous study from this laboratory, observed that dramatic improvement in clinical status and hepatic function without significant decrease in hepatic fat occurred in three chronic alcoholics with fatty cirrhosis studied when they were maintained on a diet containing essentially no protein. Their diet did contain, however, an estimated 30 to 100 mg. of choline and one vitamin capsule [6] per day.

In the present study, three similar patients were furnished with a more highly purified diet, consisting only of a solution of glucose and minerals, and studied under comparable conditions and for approximately the same length of time. Unlike the results obtained by Eckhardt and his associates, no significant improvement in hepatic function or size occurred. In fact, measurements of hepatic function became decidedly worse in one patient. Similar to their findings however, no change in hepatic fat content was apparent. In a second period of study of similar length, all conditions were kept constant except for a change from the purified to an adequate diet. The rapid improvement in hepatic function was in sharp contrast to the lack of improvement in the initial period of study. In addition, there was a considerable decrease in hepatic fat in two of the three patients, but this decrease was not apparent in the third patient until two weeks had elapsed.

It should be noted that the purified diet of the patients in this study differed not only qualitatively from the diet used by Eckhardt and his coworkers (7) but also quantitatively, as the former provided 1,600 calories or less per day, while the latter supplied almost 3,000 calories per day. R. W. consumed only about 14 calories/Kg./day, but P. F. and B. R. were able to take approximately 21 and 27 calories/Kg./day, respectively. At least in the latter two patients, this caloric intake should be sufficient for a normal sedentary person. Moreover, while R. W.'s caloric intake was low on the purified diet, it was also low on the adequate diet, although precise measurements of the latter were not made. The possibility remains that the low caloric value of the purified diet might have contributed to the failure of these patients to recover.

Because of co-existing Wernicke's syndrome, two of the patients received thiamine in addition to the purified diet with satisfactory amelioration of the abnormal neurological signs. However, no beneficial effect on the hepatic process could be attributed to the thiamine. The short term use of multivitamins in one patient was associated with a rise in B.S.P. retention and an apparent increase in hepatic fat; this single observation, however, does not justify interpretation.

The results observed attest to the efficacy of the adequate diet or of one or more of its constituents in the successful treatment of the fatty cirrhosis in these patients and suggest dietary deficiency as an important etiologic factor. The failure of these patients to improve when provided with a highly purified diet, in spite of alcohol withdrawal and rest, indicates that these factors probably played subsidiary roles, especially since improvement did occur when an adequate diet was substituted. The therapeutic component or components of an adequate diet await positive identification for man.

[6] For vitamins furnished in the present study, see Footnote 4; Eckhardt and his coworkers gave one "Dayamin" capsule daily containing thiamine hydrochloride, 5 mg.; riboflavin, 5 mg.; nicotinamide, 25 mg.; pantothenic acid, 5 mg.; pyridoxine, 1.5 mg.; ascorbic acid, 100 mg.; vitamin A, 10,000 U.S.P. units; and vitamin D, 1,000 U.S.P. units.

SUMMARY

The effects of a highly purified and subsequently of an adequate diet on hepatic function, size, and fat content were studied in three chronic alcoholics with fatty cirrhosis of the liver.

None of the three patients showed significant improvement in hepatic function, size, or fat content during the eight to ten days of a purified diet, consisting of glucose and minerals with the addition of thiamine alone in one patient and thiamine plus multivitamins in another. These results are in contrast to those obtained in a previous study in this laboratory in which three similar patients showed improvement in hepatic function and size while on a less purified diet.

After institution of an adequate diet for eight to ten days, there was improvement in hepatic function in all three patients with a decrease in hepatic fat in two. The third patient showed a significant decrease in fat in the biopsy taken after 14 days of the adequate diet.

These results suggest that the improvement observed was related to the provision of an adequate diet and that a subsidiary, if any, role was played by withdrawal of alcohol and rest in the hospital.

REFERENCES

1. György, P., The nutritional aspects of liver injury. M. Clin. North America, 1949, **33**, 1657.
2. Himsworth, H. P., Lectures on the Liver and its Diseases. Harvard University Press, Cambridge, 1950.
3. Davidson, C. S., and Gabuzda, G. J., Jr., Nutrition and disease of the liver. New England J. Med., 1950, **243**, 779.
4. Gillman, J., and Gillman, T., Perspectives in Human Malnutrition. Grune & Stratton, Inc., New York, 1951.
5. Patek, A. J., Jr., Treatment of alcoholic cirrhosis of the liver with high vitamin therapy. Proc. Soc. Exper. Biol. & Med., 1937, **37**, 329.
6. Patek, A. J., Jr., and Post, J., Treatment of cirrhosis of the liver by a nutritious diet and supplements rich in vitamin B complex. J. Clin. Invest., 1941, **20**, 481.
7. Eckhardt, R. D., Zamcheck, N., Sidman, R. L., Gabuzda, G. J., Jr., and Davidson, C. S., The effect of protein starvation and of protein feeding on the clinical course, liver function and liver histochemistry of three patients with active fatty alcoholic cirrhosis. J. Clin. Invest., 1950, **29**, 227.
8. Ducci, H., and Watson, C. J., The quantitative determination of the serum bilirubin with special reference to the prompt-reacting and chloroform-soluble types. J. Lab. & Clin. Med., 1945, **30**, 293.
9. Ley, A. B., Lewis, J. H., and Davidson, C. S., The quantitative determination of the thymol turbidity reaction of serum. J. Lab. & Clin. Med., 1946, **31**, 910.
10. Wallace, G. B., and Diamond, J. S., The significance of urobilinogen in the urine as a test for liver function. With a description of a simple quantitative method for its estimation. Arch. Int. Med., 1925, **35**, 698.
11. Harrison, G. A., Chemical Methods in Clinical Medicine: Their Application and Interpretation with the Technique of the Simple Tests. J. & A. Churchill, London, 1937, 2nd Ed.
12. Souter, A. W., and Kark, R., Quick's prothrombin test simplified by the use of a stable thromboplastin. Am. J. M. Sc., 1940, **200**, 603.
13. Chalmers, T. C., Murphy, T. L., and Taft, E. B., The incidence, character, and course of liver disease in chronic alcoholics as determined by needle biopsy. J. Clin. Invest., 1948, **27**, 528.
14. Klatskin, G., and Yesner, R., Factors in the treatment of Laennec's cirrhosis. I. Clinical and histological changes observed during a control period of bedrest, alcohol withdrawal, and a minimal basic diet. J. Clin. Invest., 1949, **28**, 723.
15. Volwiler, W., Jones, C. M., and Mallory, T. B., Criteria for the measurement of results of treatment of fatty cirrhosis. Gastroenterology, 1948, **11**, 164.
16. Buck, R. E., Observations on alcoholic fatty liver: The use of interval needle biopsy and liver function tests. J. Lab. & Clin. Med., 1948, **33**, 555.
17. Seife, M., Kessler, B. J., and Lisa, J. R., Clinical, functional, and needle biopsy study of the liver in alcoholism. Arch. Int. Med., 1950, **86**, 658.

RENAL EXCRETION OF INORGANIC PHOSPHATE IN NEWBORN INFANTS[1, 2]

By WALLACE W. McCRORY,[3] CAROLYN W. FORMAN,[4] HELEN McNAMARA, AND HENRY L. BARNETT

(From the New York Hospital and the Department of Pediatrics, Cornell University Medical College, New York City)

(Submitted for publication August 17, 1950; accepted January 28, 1952)

INTRODUCTION

Because of the important role phosphate plays in many body functions and since phosphate absorbed in excess of body needs is excreted mainly by the kidneys, detailed studies of renal excretion of phosphate have been made in dogs and in adult human subjects, especially by Pitts and his coworkers (1–3). Mechanisms involved in renal excretion of phosphate in young infants are of interest for additional reasons. The serum phosphate concentration is higher in young infants than in adults and especially so in infants receiving cow's milk of high phosphorus content (4). This high serum phosphate concentration in young infants has been attributed to a limitation in renal excretion of phosphate (4–7) and has been implicated as a factor of importance in hypocalcemic tetany of the newborn (8, 9). In addition, it has been suggested that "the paucity of phosphates in infant urines must be a great handicap to them in excreting acids and may explain many of the accepted facts about their susceptibility to an acidosis" (10). A limitation in renal excretion of phosphate in young infants is implied as underlying these physiologic consequences. However, the renal mechanisms responsible for such a limitation, if indeed it exists, have not been described. The present investigations were undertaken to examine more completely the renal excretion of phosphate in young infants.

[1] This investigation was supported in part by research grants from the National Institutes of Health, Public Health Service, and Playtex Park Research Institute.

[2] Presented in part before the Society for Pediatric Research, French Lick, Indiana, May 8, 1950.

[3] Lewis Cass Ledyard, Jr. Fellow in Pediatrics, 1949–50. Present address: Children's Hospital of Philadelphia, Philadelphia, Pa.

[4] Public Health Service Postdoctorate Research Fellow, 1949–51.

SUBJECTS AND METHODS

Twenty-two series of observations were made on a total of 19 subjects including two normal adults, one premature infant and 16 full-term newborn infants. The clinical diagnosis of neonatal tetany had been made in seven of the infants (TR, AC, EU, EN, MI, GU, and BH) because of hypocalcemia and increased neuromuscular irritability with or without convulsions. In these infants observations were performed prior to the institution of specific therapy. The remaining nine infants were normal newborn infants.

All infants received cow's milk formulas of similar caloric, calcium, and phosphorus content. In addition each received 14,000 units of Vitamin A, 2,500 units of Vitamin D, and 50 mg. of ascorbic acid daily after the sixth neonatal day. Observations were made in the morning at least three hours after feedings, and subjects were fasted throughout experimental periods. Blood specimens were drawn from scalp and peripheral veins into syringes coated with mineral oil. Hemolysis was negligible. Standard technics were used for determination of calcium in serum (11) and phosphorus in serum and urine (12).

With one exception (FL) in which only endogenous creatinine clearances were done, glomerular filtration rate (GFR) was measured in all observations on infants by the clearance of inulin, using the catheterization technic previously described (13). In 11 observations, inulin was given by continuous infusion and in seven by single injection. In observations on the two normal adults, urines were obtained by spontaneous voidings.

To determine excretion of phosphate unaffected by experimental procedures (*i.e.*, inulin infusion, parathormone injection, etc.) GFR was estimated by the endogenous creatinine clearance corrected by the creatinine: inulin clearance ratio, measured later in the same observation in all but one instance (FL). The creatinine: inulin clearance ratio is derived from the average value for each of these functions measured simultaneously during the experimental procedure. The validity of this method of estimating GFR in the absence of infusions is attested to by C_{CR}: C_{IN} mean ratio for the total observations of 1.03 (Table II).

PROCEDURES

The concentration in serum and rate of urinary excretion of inorganic phosphate was measured simultaneously with estimations of GFR under the following conditions:

1) At endogenous serum inorganic phosphate concentration in nine infants and in the two adults. The serum phosphate concentration was below the accepted upper limit of normal, 2.5 mM/L., in five infants, all of whom were normal; it was above this value in four of the infants, three of whom had neonatal tetany, and one of whom was normal.

2) In six normal newborn infants (VA, MC, GO, RI, HO, RY) whose serum phosphate concentration was elevated by a single oral dose of phosphate (0.5 to 1.5 gm. Na_2HPO_4/Kg.) administered in water by gavage. The larger dose regularly produced frequent loose stools. Serum phosphate was elevated to levels of 4.0–4.7 mM/L. in one to two hours. The change in serum phosphate during individual collection periods (15–20 minutes) did not exceed 0.7 mM/L. and was 0.3 mM or less in most periods. Hypocalcemia of varying degrees developed in association with the induced hyperphosphatemia. Though serum calcium fell to as low as 1.6 mM/L. (6.4 mg./100 ml.) no infants developed clinical signs of tetany.

Similar observations with single feedings of phosphate were made in one normal infant (RY), before and after a period of ingestion of aluminum hydroxide gel (daily dosage of 6.4 ml./Kg.) for five days.

3) Before and after intravenous injection of parathyroid extract in three normal infants (SH, GO, and FL) and in four infants with neonatal tetany (TR, AC, EN, EU).

RESULTS

A. Observations on renal phosphate excretion in relation to endogenous serum phosphate concentration

Values for serum phosphate concentrations and rates of filtration, reabsorption and excretion of phosphate together with the creatinine : inulin clearance ratios and corrected creatinine clearances in nine infants who had no preceding treatment which might affect these functions are presented in Table I.

The results show rather wide variations in individual values for rates of filtration, reabsorption, and excretion of phosphate in the nine infants. Differences in rates of excretion (and reabsorption) of phosphate were not related, however, to differences in endogenous serum phosphate concentration. In the five infants whose serum phosphate concentrations were 2.5 mM/L. or less, mean values for rates of phosphate filtered, reabsorbed, and excreted were 17.9, 15.4, and 2.5 micromols/min. compared with values of 17.4, 15.1, and 2.3 in the four infants with higher concentrations of serum phosphate. If the rate of phosphate excretion is expressed as the fraction of filtered phosphate excreted, a similar range and mean value is found in the two groups. Thus, though the two groups differ in their serum phosphate concentrations they show no related differences in filtered phosphate load or its disposition. Since filtered phosphate is the product of serum phosphate concentration and glomerular filtration rate, the fact that the groups fail to show differences in filtered phosphate implies that glomerular filtration rate is lower on the average in those infants with higher serum phosphate concentrations.

TABLE I

Renal excretion of inorganic phosphate in relation to endogenous serum phosphate concentrations in newborn infants

C_{CR}/C_{IN} represents the ratio of mean values for C_{CR} and C_{IN} for all periods (3–11) in each observation during which simultaneous measurements were made. Corrected C_{CR} is the mean C_{CR} of the pre-infusion periods (1–3) corrected by this ratio. Values given for phosphate also represent mean values for the pre-infusion periods.

Subject	Clinical status	Serum P	C_{CR}/C_{IN}	Corrected C_{CR}	Phosphate (*micromols/min.*)			C_P/C_{IN}
					Filtered	Reabsorbed	Excreted	
		mM/L.		*ml./min.*				
FL	Normal	2.2		6.3*	13.9	12.1	1.8	.13
SH	Normal	2.3	0.95	7.8	17.9	15.6	2.3	.13
GO	Normal	2.3	1.05	9.1	20.9	18.8	2.1	.10
RI	Normal	2.4	1.06	8.6	20.6	16.5	4.1	.20
GD	Normal	2.5	1.00	6.5	16.3	14.0	2.3	.14
	Mean	2.3	1.02	7.7	17.9	15.4	2.5	.14
AC	Tetany	2.7	0.88	6.7	18.1	15.9	2.2	.12
BO	Normal	2.8	1.02	6.9	19.3	17.1	2.2	.11
EU	Tetany	2.9	0.90	6.2	18.0	15.4	2.6	.14
EN	Tetany	2.9	1.00	4.9	14.2	12.0	2.2	.15
	Mean	2.8	0.95	6.2	17.4	15.1	2.3	.13

* In this single instance in which no inulin clearance was done, the endogenous creatinine clearance was corrected by the mean creatinine : inulin ratio (1.03) determined in observations on the other infants (see Table II).

TABLE II

Relation of endogenous serum phosphate concentrations to glomerular filtration rate in newborn infants

Subject	Clinical data	Sex	Age	Weight	S. A.	Serum P	C_{IN}	C_{CR}	C_{IN}/C_{CR}
			days	*Kg.*	M^2	*mM/L.*	*ml./min.*	*ml./min.*	
VA	Normal	M	10	3.46	.23	2.1	6.3	6.7	1.06
MC	Normal	M	14	4.10	.27	2.3	8.4	9.3	1.11
SH	Normal	M	10	3.25	.22	2.3	8.5	8.1	0.95
GO	Normal	M	11	3.60	.24	2.3	8.6	9.0	1.05
RI	Normal	M	14	3.78	.25	2.4	7.1	7.5	1.06
GD	Normal	F	6	3.30	.23	2.5	6.5	6.5	1.00
Mean			10.8	3.60	0.24	2.3	7.6	7.9	1.04
TR	Tetany	F	8	3.00	.21	2.7	4.2	3.7	0.88
AC	Tetany	M	7	3.37	.23	2.7	6.7	5.9	0.88
BO	Normal	F	16	4.48	.29	2.8	6.5	6.6	1.02
EU	Tetany	M	12	3.83	.25	2.9	7.0	6.3	0.90
EN	Tetany	M	5	3.20	.22	2.9	5.2	5.2	1.00
MI	Tetany	F	19	4.20	.27	3.0	6.5	7.8	1.20
GU	Tetany	M	14	3.72	.25	3.0	7.5	8.3	1.11
BH	Tetany	F	8	3.20	.22	3.1	4.9	5.7	1.16
Mean			11.1	3.63	0.24	2.9	6.1	6.2	1.02

The values for glomerular filtration rates and endogenous serum phosphate concentrations in 14 of the infants are presented in Table II, together with pertinent clinical data. Inulin clearances for six infants, whose endogenous serum phosphate concentrations were known but whose control rates of phosphate excretion were not measured, are included in this table.

The mean value for inulin clearances in the six infants whose serum phosphate concentrations were 2.5 mM/L. or less was 7.6 ml./min. compared with a value of 6.1 in eight infants with serum phosphate concentrations above 2.5 mM/L. The two groups have the same mean value for surface area, 0.24 M^2; hence the difference in rate of glomerular filtration is not a reflection of differences in surface area.

B. Effect of induced acute elevation of serum phosphate on renal mechanisms of phosphate excretion

Since we failed to observe a relationship between the rate of tubular reabsorption of phosphate and endogenous serum phosphate concentration in these infants, the study of the maximal or limiting

TABLE III

Effect of rapid elevation of serum phosphate concentration on renal excretion of phosphate in an infant

Subject	Period	Elapsed time	Serum P	C_{IN}	C_{CR}	P (*micromols/min.*)			C_P	C_P/C_{IN}
						Filtered	Reabsorbed	Excreted		
		min.	*mM/L.*	*ml./min.*	*ml./min.*				*ml./min.*	
RI	1	0–30	2.5	8.3*	8.8	20.8	16.6	4.2	1.68	.20
2 weeks	2	31–58	2.4	8.8*	9.3	21.1	17.1	4.0	1.67	.19
3.78 Kg.		Single injection of inulin at 65 min. 1.8 gm. Na_2HPO_4 at 72 min.								
	3	121–143	3.5	7.3	7.7	25.6	14.3	11.3	3.23	.44
	4	144–163	4.1	7.1	7.6	29.1	13.7	15.4	3.76	.53
	5	164–185								
	6	186–205	4.4	6.8	7.6	29.9	12.4	17.5	3.98	.59
	7	206–225	4.2	7.5	7.2	31.5	13.9	17.6	4.19	.56

* Values calculated from endogenous creatinine clearance corrected on the basis of subsequently determined creatinine:inulin clearance ratio.

rate of tubular reabsorption of phosphate was attempted.

Pitts and Alexander (1) demonstrated a relatively stable limiting rate of phosphate reabsorption in dogs when sufficient inorganic phosphate was infused to increase rate of filtration of phosphate well above the maximal rate of tubular reabsorption of phosphate. Assuming that differences in maximal or limiting rates of phosphate reabsorption (as distinguished from rate of reabsorption at endogenous serum phosphate concentrations) could exist in these infants, observations on infants were made before, during, and after rapid elevation of serum phosphate.

The protocol of a typical observation (RI) is shown in Table III. The calculated rate of reabsorption did not change significantly though a marked increase in rate of filtration of phosphate occurred with elevation of serum phosphate. The increase in rate of filtration of phosphate was consequently associated with an equivalent increase in phosphate excretion. The significant finding was the absence of an increase in rate of tubular reabsorption of phosphate as serum phosphate was elevated. The pattern of response in six normal infants (VA, MC, GO, RI, HO, RY) is shown graphically in Figure 1. All values were corrected to 1.73 M^2 for comparison. The linear relationship between increasing rates of filtration and excretion of phosphate defines a limited rate for phosphate reabsorption. The finding of a limited or maximal rate for phosphate reabsorption in these infants is in agreement with the observations of Pitts and Alexander (1) and Harrison and Harrison (14) in adult human subjects and dogs. The maximal rate for phosphate reabsorption at elevated serum phosphate concentrations in these infants receiving cow's milk of high phosphate content did not differ significantly from the rate of reabsorption at their endogenous concentrations of serum phosphate. Since the rate of phosphate reabsorption is apparently independent of rapid increases in serum phosphate, the endogenous value for tubular reabsorption under these circumstances is identical with the physiologic maximal or limiting rate of phosphate reabsorption.

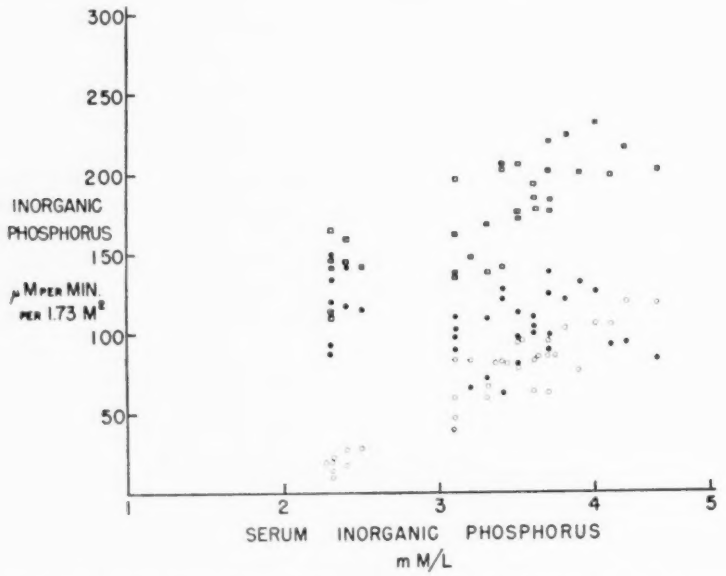


FIG. 1. EFFECT OF RAPID ELEVATION OF SERUM PHOSPHATE CONCENTRATION ON RENAL EXCRETION OF PHOSPHATE IN SIX INFANTS

Symbols designate values for rates of filtration (open squares), reabsorption (dots) and excretion (open circles) of phosphate.

C. Effect of reduced intestinal absorption of phosphate on renal excretion of inorganic phosphate

The administration of aluminum hydroxide gel has been shown to reduce markedly the amount of dietary phosphorus available for intestinal absorption and to decrease urinary excretion of phosphate (15, 16). Accordingly this drug induces a state which may be considered comparable to that obtaining with low dietary intake of phosphate. Advantage was taken of this effect to examine the accompanying changes in renal excretion of inorganic phosphate in one normal infant (RY). Values for serum phosphate, glomerular filtration rate and renal excretion of phosphate observed in subject RY before and after he had received aluminum hydroxide gel for five days are given in Table IV. In addition after control periods in each observation, serum phosphate was elevated by phosphate ingestion. Although serum phosphate, glomerular filtration rate and consequently filtered phosphate are quite comparable in the two observations, *a striking difference is observed in phosphate reabsorbed and excreted.* In the second observation at endogenous serum phosphate concentrations, almost no phosphate was being excreted into the urine, which in the presence of an unchanged rate of filtration of phosphate represents a marked increase in rate of tubular reabsorption of phosphate. At both endogenous and elevated concentrations of serum phosphate the rate of tubular reabsorption is markedly increased following aluminum hydroxide gel. This change in rate of tubular reabsorption has reduced by over 50 per cent the rate at which a large load of phosphate is excreted.

These data indicate that reduction in dietary phosphorus is accompanied by decreased phosphate excretion due to increased tubular reabsorption of phosphate with no change in serum phosphate concentration.[5] Furthermore, it appears that the limiting rate of phosphate reabsorption which

[5] It should be pointed out that these changes in reabsorption have been demonstrated to be independent only of the fasting endogenous serum phosphate concentration. It is possible that reduction in dietary intake might influence diurnal variations in serum phosphate (16), which in turn might be concerned in the factors regulating reabsorption.

TABLE IV

Renal excretion of phosphate at endogenous and rapidly elevated serum phosphate concentrations before (A) and after (B) administration of aluminum hydroxide gel

	Subject	Period	Elapsed time	Serum P	C_{IN}	C_{CR}	P (*micromols/min.*)			C_P	C_P/C_{IN}
							Filtered	Reabsorbed	Excreted		
			min.	*mM/L.*	*ml./min.*	*ml./min.*				*ml./min.*	
A	RY 3 mos. 2.10 Kg. Normal premature 3/17/50	1	0–24	2.3	4.5*	4.5	10.4	8.2	2.2	0.96	.21
		2	25–57	2.3	4.6*	4.6	10.6	8.7	1.9	0.83	.18
			Continuous infusion of inulin at 48 min. 0.63 gm. Na_2HPO_4 at 67 min.								
		3	117–136	3.2	4.3	4.3	13.8	6.1	7.7	2.41	.56
		4	137–156	3.4	3.9	3.7	13.3	5.8	7.5	2.21	.57
		5	157–176	3.5	4.6	4.7	16.1	7.4	8.7	2.49	.54
		6	177–196	3.3	3.9	3.8	12.9	6.6	6.3	1.91	.49
B	RY 2.02 Kg. 3/24/50	1	0–20	2.4	4.8*	4.8	11.5	11.4	0.1	0.04	.01
		2	21–39	2.4	5.7*	5.8	13.7	13.6	0.1	0.04	.01
			Continuous infusion of inulin at 34 min. 0.61 gm. Na_2HPO_4 at 41 min.								
		3	118–137	3.8	4.3	4.2	16.3	12.8	3.5	0.92	.21
		4	158–178	3.6	3.8	3.9	13.7	10.3	3.4	0.94	.25
		5	179–198	3.4	4.0	4.0	13.6	10.3	3.3	0.97	.24

* Values calculated as explained in Table III.

is demonstrable under given conditions is a physiologic reflection of homeostatic adjustments and is not a specific tubular function independent of hormonal and metabolic influence.

D. Effect of parathyroid extract on renal excretion of phosphate

The effect of parathyroid extract on serum phosphate concentration, glomerular filtration rate and renal excretion of phosphate was consistent in all observations made on each of seven infants (SH, GO, FL, TR, AC, EU, EN). The protocol of a typical response is given in Table V (EU). Data from an infant (GU) not given parathyroid extract but treated similarly in every other respect are included in Table V as a control observation. A significant increase in phosphate excretion regularly followed the intravenous injection of 15 units of parathyroid extract. In a single observation (FL), no response followed 10 units of parathyroid extract given intravenously. The magnitude of the response was independent of the endogenous serum phosphate concentration. As can be seen from Table V, the observed increases in phosphate excretion could not be accounted for by increases in glomerular filtration rate or serum phosphate concentration but could be attributed only to significant decreases in rate of phosphate reabsorption. The changes observed in phosphate reabsorption and excretion following parathyroid extract exceeded spontaneous or diurnal changes or experimental variations as can be judged from the control observation presented.

These observations support the findings of Harrison and Harrison (14) and Michie and Shorey (17) that one effect of parathyroid extract administered intravenously is to increase renal excretion of phosphate by decreasing the rate

TABLE V

A. Effect of intravenous injection of parathyroid extract on renal excretion of phosphate
B. Control observation

	Subject	Period	Elapsed time	Serum P	C_{IN}	C_{CR}	P (*micromols/min.*)			C_P	C_P/C_{IN}
							Filtered	Reabsorbed	Excreted		
			min.	*mM/L.*	*ml./min.*	*ml./min.*				*ml./min.*	
A	EU 12 days 3.83 Kg. Tetany	1	0–26	2.9	6.2*	5.6	18.0	15.5	2.5	0.86	.14
			Continuous infusion of inulin at 32 min.								
		2	27–50	2.8	7.7*	6.9	21.6	18.2	3.4	1.21	.16
		3	51–74	2.7	8.4*	7.6	22.7	19.1	3.6	1.33	.16
		4	75–115	2.7	6.2	6.1	16.7	13.5	3.2	1.19	.19
			PTH 15 units at 117 min.								
		5	116–134	2.6	6.5	5.5	16.9	12.3	4.6	1.77	.27
		6	135–154	2.5	7.3	6.5	18.3	12.9	5.4	2.16	.30
		7	155–174	2.4	7.2	6.3	17.3	12.6	4.7	1.96	.27
		8	175–194	2.4	8.4	7.3	20.2	14.8	5.4	2.25	.27
B	GU 14 days 3.72 Kg.	1	0–11	3.0	7.9*	8.3	23.7	18.0	5.7	1.90	.24
		2	12–36	3.0	8.6*	9.0	25.8	19.7	6.1	2.03	.24
		3	37–55	3.1	8.0*	8.4	24.8	18.9	5.9	1.90	.24
			Continuous infusion of inulin at 71 min.								
		4	56–96	3.0	6.8*	7.2	20.4	15.8	4.6	1.53	.23
		5	97–125	2.9	8.0*	8.4	23.2	18.0	5.2	1.79	.22
		6	126–145	2.8	7.9	8.6	22.1	16.9	5.2	1.86	.24
		7	146–165	2.7	6.9	7.2	18.6	14.2	4.4	1.63	.24
		8	166–185	2.7	7.8	7.9	21.1	16.4	4.7	1.74	.22

* Values calculated as explained in Table III.

TABLE VI

Comparison of renal excretion of phosphate at endogenous serum phosphate concentrations in infants, children, and adults

Subjects	Number	GFR	Serum P conc.	Phosphate (*micromols/min./1.73 M²*)			P Clearance	C_P/C_{IN}
				Filtered	Reabsorbed	Excreted		
		ml./min./1.73 M²	*mM/L.*				*ml./min./1.73 M²*	
Newborn infants (*cow's milk*)	5	55.5	2.34	129.0	111.0	18.0	7.7	.14
Newborn infants* (*cow's milk*)	3	38.2	2.55	97.4	79.2	18.2	7.1	.19
Newborn infants† (*human milk*)	9	—	1.98	—	—	6.0	3.0	—
Children*	6	116.6	1.65	192.4	167.0	25.4	15.4	.13
Adults	2	133.3	1.15	153.3	144.7	8.6	7.5	.06
Adults‡	10	113.2	1.04	117.7	113.3	4.4	4.2	.04
Adults§	4	—	1.03	—	—	13.3	12.9	—

* Richmond, Kravitz, Segar, and Waisman (7).
† Dean and McCance (6).
‡ Lambert, van Kessel, and Leplat (22).
§ Ollayos and Winkler (23).

of tubular reabsorption. This effect has been disputed by others (18, 19). These data also confirm the unpublished observations of Crawford, Osborne, Talbot and Terry (20) that in newborn infants, an increased rate of renal phosphate excretion occurs in response to exogenous parathyroid extract.

E. Comparison of renal excretion of phosphate in newborn infants and adults

The finding of Dean and McCance (6) that phosphate clearances are much lower in newborn infants than in adults has been interpreted as evidence of a limitation of the infant's ability to excrete phosphate in comparison with adults. Table VI shows interesting quantitative differences between infants and normal adults in the mechanisms for renal excretion of inorganic phosphate. Surface area appears to provide the best standard of reference for comparing renal functions in subjects of different sizes (21). Mean values for rates of filtration, reabsorption, and excretion of phosphate and phosphate clearances corrected to 1.73 M^2 observed in the five infants whose endogenous serum phosphate concentrations ranged from 2.1 to 2.5 mM/L. are included in Table VI. These data are chosen for comparison with adult values because the serum phosphate concentrations in these infants represent the normal range for infants receiving cow's milk. Included in Table VI are mean values for ten normal adults reported by Lambert, van Kessel, and Leplat (22); for three normal adults and one with mild essential hypertension reported by Ollayos and Winkler (23);[6] for six older children and three newborn infants reported by Richmond, Kravitz, Segar and Waisman (7); and for nine infants observed by Dean and McCance (6); together with two normal adults studied by the present authors.

It is apparent from Table VI that, on the basis of surface area, newborn infants *can* excrete phosphate at a higher rate than fasting adults even though glomerular filtration rate in the infants is less than one-half that of adults. In the infants reported here higher rates of excretion are due to higher serum phosphate concentrations and lower rates of tubular reabsorption of phosphate. Phos-

[6] Ollayos and Winkler (23) have demonstrated a marked diurnal variation in rate of phosphate excretion. Although it is not known whether infants show similar fluctuations, for purposes of comparison the values taken from Ollayos and Winkler represent their control periods (1 to 4) extending from 65 to 360 minutes after the observations were begun (about 7:00 a.m.). This range of time covers the period during which observations on infants and adults were made in the present study.

It is not possible to compare the values given in Table VI with those on adults reported by Dean and McCance (6). They confirmed the finding of diurnal variations in adults but did not give values for individual periods for most of their adult subjects. In their subjects Nos. 14 and 15, for whom forenoon values were given, the phosphate clearances were 6.5 and 6.6 ml./min., respectively, which are in the same range as those of Richmond, Kravitz, Segar, and Waisman (7), Lambert, van Kessel, and Leplat (22), and Ollayos and Winkler (23), and those reported here.

phate clearances, however, are in the same broad range.

These quantitative differences in renal excretion of inorganic phosphate between newborn infants receiving cow's milk and fasting adults should not be interpreted as indicating differences in renal function *per se*. Gardner and his associates (4) have demonstrated that phosphate excretion and consequently phosphate clearances vary markedly with phosphate intake. It is apparent from the data of Dean and McCance (6) (Table VI) obtained from infants receiving human milk (4) that under other circumstances newborn infants *may* not be excreting phosphate at rates as high as adults.

DISCUSSION

Renal retention of phosphate implies either abnormally decreased filtration or increased tubular reabsorption of phosphate or a combination of both.

The occurrence of elevated serum phosphate concentrations in young infants receiving cow's milk of high phosphate content has been attributed to a limitation in renal excretion of phosphate due either to renal immaturity *per se* or insufficient parathyroid activity or a combination of both. In a group of infants on similar intakes of phosphate we have observed no apparent relationship between rate of phosphate excretion and serum phosphate concentrations.

Infants with widely varying serum phosphate concentrations were found to be filtering, reabsorbing, and excreting phosphate at rates which were in general comparable. Insofar as the rate of phosphate reabsorption is a measure of parathyroid activity, these data indicate that differences in endogenous parathyroid activity do not account for differences in serum phosphate concentrations in these infants. On the other hand, a relationship does seem to exist between elevation of serum phosphate and lower rates of glomerular filtration. It would appear, therefore, that infants receiving high intakes of phosphate, who have low rates of glomerular filtration, excrete the required amount of phosphate by elevation of serum phosphate concentration rather than by further lowering of the rate of tubular reabsorption, which in relation to phosphate filtered is less than that observed in adults.

Why infants with lower rates of glomerular filtration do not have lower rates of tubular reabsorption which would permit the same excretion at lower serum phosphate concentration, cannot be answered at present. Conceivably, failure to further reduce the rate of tubular reabsorption could be due, as suggested by Dean and McCance (6), to inherent renal glomerular-tubular imbalance or to either inadequate parathyroid activity or decreased responsiveness to parathyroid hormone. We observed no difference in the response of infants with different serum phosphate concentrations to similar amounts of exogenous parathyroid extract. If this circumstance were to be attributed to altered parathyroid function, it would appear to represent inadequate compensatory hyperparathyroidism (24, 25) rather than absolute hypoparathyroidism since the fraction of filtered phosphate excreted by infants is higher than that in adults (Table III) and is similar in all infants (Table I).

Intestinal absorption of phosphate directly affects rate of renal excretion of phosphate as demonstrated by observations made in a single infant before and after a five-day period of reduced intestinal absorption of phosphate. After aluminum hydroxide gel, phosphate practically disappeared from the urine although there was no change in endogenous serum phosphate concentration [5] or glomerular filtration rate. Similarly, the small quantities of phosphate in urines of infants on breast milk may be considered a consequence of the low dietary phosphate intake and high metabolic demand. It is only under such circumstances that a low renal excretion of phosphate would, by reducing the rate of excretion of buffer base (26), influence the susceptibility of young infants to acidosis. Perhaps of greater interest is the fact that the "ability" of a single infant to excrete a large load of ingested phosphate was markedly decreased due wholly to increased tubular reabsorption following a period of low intake of phosphate. The "ability" of an infant to excrete phosphate is thus markedly influenced by dietary intake of phosphate as well as by the degree of "maturity" of renal function.

It is difficult to attempt to compare infants with adults in respect to their "ability" to excrete phosphate. Under the conditions of the present observations and with values corrected on the basis of surface area, these infants receiving cow's milk

excreted phosphate at higher rates than adults and had values for phosphate clearances in the same range. This observation is in disagreement with the conclusions of Dean and McCance (6). However, their observations were made on infants receiving breast milk. "Average values" for clearances of electrolytes cannot be interpreted in the same sense as clearances representing discrete kidney functions. Differences in intake, retention, hormonal status, metabolic demand and perhaps other factors complicate the problem so that comparisons of "average values" for electrolyte clearances in infants and adults have limited significance.

SUMMARY AND CONCLUSIONS

The concentration in serum and the rate of urinary excretion of inorganic phosphate were measured simultaneously with estimations of glomerular filtration rate in 17 infants and in two adults. The newborn infants included two groups defined by the presence of a normal or elevated endogenous serum phosphate concentration. Seven of the infants in the latter group had neonatal tetany.

Observations were made under one or more of the following conditions: *1*) at endogenous concentrations of serum phosphate; *2*) at elevated concentrations of serum phosphate resulting from oral ingestion of phosphate; *3*) before and after a period of decreased intestinal absorption of phosphates achieved by ingestion of aluminum hydroxide gel; and *4*) before and after the intravenous administration of parathyroid extract.

The observations are interpreted as indicating that: *1*) differences in serum phosphate concentrations in normal infants on similar diets may reflect differences in rates of glomerular filtration but cannot be related to differences in rates of renal excretion or tubular reabsorption of phosphate; *2*) insofar as the rate of phosphate reabsorption is a measure of parathyroid activity, differences in endogenous parathyroid activity do not account for differences in serum phosphate concentrations in these infants; *3*) a maximal rate of tubular reabsorption of phosphate can be demonstrated in young infants, and infants on cow's milk are reabsorbing phosphate at this maximal rate; *4*) changes in this maximal rate of tubular reabsorption of phosphate may be evoked by changes in intestinal absorption of phosphate; *5*) the renal response of young infants to parathyroid extract given intravenously is similar to that of adults; and *6*) although infants receiving cow's milk can excrete phosphate at a higher rate per unit surface area than fasting adults, so many non-renal factors influence the rate of renal phosphate excretion that comparisons between infants and adults do not afford a measure of their comparative renal "ability" to excrete phosphate.

ACKNOWLEDGMENTS

We are indebted to Flora Hurwitz, R. N., for nursing and technical assistance.

REFERENCES

1. Pitts, R. F., and Alexander, R. S., The renal reabsorptive mechanism for inorganic phosphate in normal and acidotic dogs. Am. J. Physiol., 1944, **142**, 648.
2. Ayer, J. L., Schiess, W. A., and Pitts, R. F., Independence of phosphate reabsorption and glomerular filtration in the dog. Am. J. Physiol., 1947, **151**, 168.
3. Pitts, R. F., Lotspeich, W. D., Schiess, W. A., and Ayer, J. L., The renal regulation of acid-base balance in man. I. The nature of the mechanism for acidifying the urine. J. Clin. Invest., 1948, **27**, 48.
4. Gardner, L. I., MacLachlan, E. A., Pick, W., Terry, M. L., and Butler, A. M., Etiologic factors in tetany of newly born infants. Pediatrics, 1950, **5**, 228.
5. McCance, R. A., and von Finck, M. A., The titratable acidity, pH, ammonia and phosphates in the urines of very young infants. Arch. Dis. Child., 1947, **22**, 200.
6. Dean, R. F. A., and McCance, R. A., Phosphate clearances in infants and adults. J. Physiol., 1948, **107**, 182.
7. Richmond, J. B., Kravitz, H., Segar, W., and Waisman, H. A., Renal clearance of endogenous phosphate in infants and children. Proc. Soc. Exper. Biol. & Med., 1951, **77**, 83.
8. Bakwin, H., Pathogenesis of tetany of the newborn. Am. J. Dis. Child., 1937, **54**, 1211.
9. Bakwin, H., Tetany in newborn infants; relation to physiologic hypoparathyroidism. J. Pediat., 1939, **14**, 1.
10. McCance, R. A., Renal physiology in infancy. Am. J. Med., 1950, **9**, 229.
11. Clark, E. P., and Collip, J. B., A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem., 1925, **63**, 461.

12. Fiske, C. H., and SubbaRow, Y., Colorimetric determination of phosphorus. J. Biol. Chem., 1925, **66**, 375.
13. Barnett, H. L., Hare, K., McNamara, H., and Hare, R., Measurement of glomerular filtration rate in premature infants. J. Clin. Invest., 1948, **27**, 691.
14. Harrison, H. E., and Harrison, H. C., Renal excretion of inorganic phosphate in relation to action of vitamin D and parathyroid hormone. J. Clin. Invest., 1941, **20**, 47.
15. Freeman, S., and Freeman, W. M. C., Phosphorus retention in children with chronic renal insufficiency. The effect of diet and of the ingestion of aluminum hydroxide. Am. J. Dis. Child., 1941, **61**, 981.
16. Shorr, E., and Carter, A. C., Aluminum gels in the management of renal phosphatic calculi. J. A. M. A., 1950, **144**, 1549.
17. Michie, A. J., and Shorey, J. M., Parathyroid extract hyperphosphaturia in hypoparathyroidism. Federation Proc., 1950, **9**, 88.
18. Jahan, I., and Pitts, R. F., Effect of parathyroid on renal tubular reabsorption of phosphate and calcium. Am. J. Physiol., 1948, **155**, 42.
19. Hogben, C. A. M., and Bollman, J. L., Renal reabsorption of phosphate: normal and thyroparathyroidectomized dog. Am. J. Physiol., 1951, **164**, 670.
20. Crawford, J. D., Osborne, M. M., Jr., Talbot, N. B., and Terry, M. L., Parathyroid glands and phosphorus homeostasis. Cited in reference 4 above.
21. Rubin, M. I., Bruck, E., and Rapoport, M., Maturation of renal function in childhood: clearance studies. J. Clin. Invest., 1949, **28**, 1144.
22. Lambert, P. P., van Kessel, E., and Leplat, C., Etude sur l'élimination des phosphates inorganiques chez l'homme. Acta. med. Scandinav., 1947, **128**, 386.
23. Ollayos, R. W., and Winkler, A. W., Urinary excretion and serum concentration of inorganic phosphate in man. J. Clin. Invest., 1943, **22**, 147.
24. Kaplan, E., Parathyroid gland in infancy. Arch. Path., 1942, **34**, 1042.
25. Albright, F., and Reifenstein, E. C., Jr., The Parathyroid Glands and Metabolic Bone Disease. Williams & Wilkins Co., Baltimore, 1948.
26. Pitts, R. F., Acid-base regulation by the kidney. Am. J. Med., 1950, **9**, 356.

THE EXCHANGEABLE POTASSIUM CONTENT OF NORMAL WOMEN [1, 2]

By JERRY K. AIKAWA,[3] GEORGE T. HARRELL, AND BERNARD EISENBERG

(From the Department of Internal Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, North Carolina)

(Submitted for publication September 17, 1951; accepted February 1, 1952)

A method, utilizing a radioactive isotope (K^{42}), for the measurement of the exchangeable potassium content of the body has been recently reported (1). That study was performed on a relatively homogeneous group of 30 young male subjects. Because of differences in physical characteristics and endocrine functions, the relative body potassium content in the two sexes might be dissimilar. The normal range in healthy young women has not been established. In the present study, the exchangeable potassium content in a homogeneous group of 20 healthy young adult women was determined and an attempt was made to correlate it with body weight and urinary creatinine excretion.

MATERIAL AND METHODS

The subjects were 20 healthy nurses, medical students, and laboratory technicians between the ages of 19 and 32 years. They were ambulatory and continued with their usual activities during the period of the study. Two groups consisting of nine and 11 individuals were studied two weeks apart. The subjects were in various stages of their menstrual cycle.

Experimental procedure: Each subject was given an intravenous injection of radioactive potassium [4] by calibrated syringe in the morning between 6:30 and 9:00 a.m. All urine specimens until 7:00 a.m. the next morning were collected and pooled, and this collection of urine was measured for the excretion of K^{42}. Specific activity of the urine was determined on two spot samples collected at 8:00 and 9:00 a.m. on the day after injection. Preliminary studies confirmed the observations of Corsa and associates (1) that the specific activity of K^{42} in the urine had reached an equilibrium by 24 hours. No restrictions were placed on activity or on the intake of food, water or salt.

Preparation and administration of radioactive potassium: The shipments of K^{42} were treated in the manner described by Corsa and his co-workers (1). One and a half milliequivalents of potassium chloride solution, containing 100 microcuries of K^{42}, were injected intravenously.

Measurement of radioactivity: The activity of the urine specimens was determined with a dipping tube and a scaling circuit. Counts were made to 1% accuracy. All counting rates were at least ten times background, and were usually in the range of 500 to 3,000 per minute. At these counting rates, no correction for coincidence was necessary. All determinations were corrected for decay.

Chemical determinations: The total potassium concentration in the urine was determined by flame photometry, using a method which has been described previously (2). Urinary creatinine concentration was determined by the method of Bonsnes and Taussky (3).

Calculation of exchangeable potassium: The following formula was used to calculate the value for the exchangeable potassium content of the body:

$$K_e = \frac{K_i^{42} - K_u^{42}}{\frac{K_u^{42}}{K_u^{39}}}$$

K_e = quantity of exchangeable potassium in milliequivalents.
K_i^{42} = quantity of radiopotassium administered (arbitrary units).
K_0^{42} = quantity of radiopotassium excreted in the urine in the pooled specimen.
K_u^{42} = concentration of radiopotassium in the urine samples.
K_u^{42}/K_u^{39} = mean specific activity of two spot specimens.

RESULTS

The measurements are summarized in Table I. The mean value for the K_e was 1776 meq. with a standard deviation of 229. The mean value for K_e/Wt. was 31.5 meq./Kg. with a standard deviation of 2.9. Although there was a considerable latitude in the values for K_e (1374 – 2164 meq.) and K_e/Wt. (25.1 – 35.9 meq./Kg.), these ranges were less than those reported in normal young males (1). A slight correlation was found by visual inspection between K_e to Wt., K_e/Wt. to creatinine coefficient (Figure 1), and K_e to creatinine. Calculation of the data in relation to surface area, which would tend to correct out the variation

[1] This work was supported (in part) by the U. S. Atomic Energy Commission under a contract with the Bowman Gray School of Medicine and (in part) by a research grant from the American Heart Association.

[2] Technical assistance by Miss Eloise Rhoades is gratefully acknowledged.

[3] Research Fellow of the American Heart Association.

[4] The K^{42} used was supplied by the National Laboratory, Oak Ridge, Tennessee, on allocation from the U. S. Atomic Energy Commission.

TABLE I

Exchangeable potassium content of normal young adult women

Subjects	Age (yrs.)	Height (cm.)	Exchangeable potassium (meq.)	Range† (% of mean)	Weight (Kg.)	K_e/Wt. (meq./Kg.)	K^{40} Excretion (% dose)	Creatinine	
								(mg./Kg.)	(mg.)
Pa	26	171	2,164	8.87	62.7	34.5	3.11	22.3	1,400
Mc	24	171	2,087	9.67	60.9	34.3	4.20	20.5	1,248
St*	25	171	2,055	0.92	59.1	34.8	4.10	22.0	1,300
Ke	23	170	2,034	0.49	61.4	33.1	2.90	21.6	1,322
Ca	21	166	1,993	2.40	55.1	35.9	4.00	22.7	1,250
Fo*	25	170	1,989	2.80	61.4	32.4	4.92	23.3	1,431
Ye	19	170	1,959	3.87	56.4	34.7	3.59	12.8	718
Wh	24	163	1,909	2.93	60.9	31.3	2.36	28.6	1,739
Mi	25	173	1,786	19.50	59.1	30.2	2.63	24.1	1,427
Sw	32	163	1,721	2.55	68.6	25.1	4.83	18.4	1,261
Ho	21	169	1,677	4.20	48.6	34.5	3.19	22.5	1,092
Ru	21	170	1,675	9.61	60.5	27.7	2.81	17.1	1,034
Ro	23	168	1,648	6.70	53.2	31.0	3.40	21.7	1,154
Mo	32	163	1,647	15.60	50.9	32.4	5.39	21.5	1,094
Ha	23	160	1,630	4.41	55.5	29.4	2.02	21.8	1,209
Hu	22	159	1,589	8.50	54.5	29.2	4.43	23.4	1,273
Ga	24	163	1,563	11.80	55.9	28.0	2.45	21.6	1,208
Hr	25	157	1,524	0.85	47.0	32.4	2.79	22.9	1,095
Rh	23	166	1,489	19.80	48.6	30.6	3.68	29.2	1,420
Wa*	25	157	1,374	0.80	47.3	29.0	3.61	23.0	1,088
Mean	24.2	166	1,776	6.81	56.4	31.5	3.52	22.0	1,237
Range	19–32	157–173	1,374–2,164	0.49–19.80	47.0–68.6	25.1–35.9	2.02–5.39	12.8–29.2	718–1,739

* The menstrual period had just started in these three individuals when this study was made.
† Range of exchangeable potassium values based on urine specific activities, as % of the mean.

due to obesity, did not result in better correlation of K_e to creatinine.

DISCUSSION

The women in the present study were of an age group comparable to that of men investigated by Corsa and associates (Table II). The men were roughly 12 cm. taller and 16 Kg. (approximately 30%) heavier. The most striking difference between the two sexes was in the mean value for K_e/Wt., which, in the females, was ⅔ of that found in males. This difference is apparently due to the greater relative fat content in women. The high range of the creatinine coefficient indicates

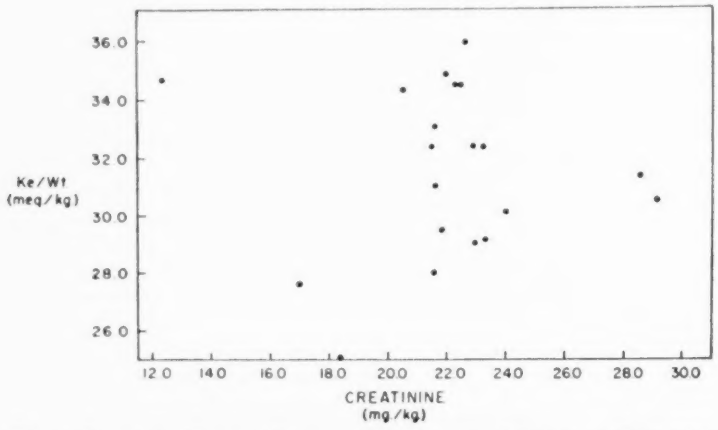


FIG. 1 CORRELATION BETWEEN EXCHANGEABLE POTASSIUM CONTENT AND CREATININE EXCRETION IN NORMAL YOUNG ADULT WOMEN

TABLE II

Exchangeable potassium content of normal young adult males (1)

	Age (*yrs.*)	Height (*cm.*)	Exchangeable potassium (*meq.*)	Weight (*Kg.*)	K_e/Wt. (*meq./Kg.*)	Creatinine (*mg.*)
Mean	25.8	177.5	3,402	72.6	46.3	1,724*
Range	21–30	162–198	2,770–4,470	57.0–125.5	35.6–53.6	

All values are based on determinations in 30 subjects.
* Mean of five determinations.

the variability in their relative muscle mass. The lack of correlation between K_e and creatinine in women indicates the variability in the proportion of potassium containing tissues other than muscle to muscle mass among individuals of this sex.

It is recognized that an incomplete urine collection may lead to a false low creatinine excretion and a false high K_e. In the present study this error is considered to be minimal, since all subjects were cooperative volunteers. In men, there appears to be a good correlation between K_e and urinary creatinine excretion, so that a fair estimate of K_e is possible from the latter measurement. Such apparently is not the case in females. It is possible that a more detailed study of a larger series of women with due considerations for the variations in age, body build, and the stage of the menstrual cycle may establish a closer correlation of K_e with other anthropometric measurements. It would appear, from the data to date, that in any study in women, the definition of alterations in the exchangeable potassium content of the body should depend on serial determinations in the same individual.

SUMMARY

The mean exchangeable potassium content in a homogeneous group of 20 normal young women was found to be 31.5 meq./Kg. This value was considerably lower than the value in normal young men. Only slight correlation was found with body weight or urinary creatinine excretion.

REFERENCES

1. Corsa, L., Jr., Olney, J. M., Steenburg, R. W., Ball, M. R., and Moore, F. D., The measurement of exchangeable potassium in man by isotope dilution. J. Clin. Invest., 1950, **29**, 1280.
2. Aikawa, J. K., Fluid volumes and electrolyte concentrations in normal rabbits. Am. J. Physiol., 1950, **162**, 695.
3. Bonsnes, R. W., and Taussky, H. H., On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., 1945, **158**, 581.

THE DISTRIBUTION OF THE THIOSULFATE ION WITH RESPECT TO NORMAL HUMAN SERUM AND RED BLOOD CELLS AND ITS APPLICATION TO "EXTRACELLULAR FLUID" DETERMINATION [1]

By HENRY J. KOWALSKI AND DAVID D. RUTSTEIN

WITH THE TECHNICAL ASSISTANCE OF RUTH I. SCHAUFUS AND JOAN M. SHOULBERG

(From the Department of Preventive Medicine, Harvard University Medical School, Boston, Mass.)

(Submitted for publication July 7, 1951; accepted February 5, 1952)

Fluid compartment measurements are useful procedures in studying edema, dehydration, and changes in cellular permeability. The general principles involved in such measurements have recently been discussed by Levitt and Gaudino (1). Substances used in the intact animal or in man should fulfill a number of conditions, among them being "uniform distribution throughout the compartment." For "extracellular fluid" estimations, this condition implies equal concentration of the substance throughout all of the intra- and extravascular fluid outside of body cells. If the material employed is physically or chemically bound by non-diffusible components of serum, intravascular concentration will exceed extravascular concentration, and uniform distribution will not occur.

Recent work performed in part at this laboratory shows that the thiocyanate ion, heretofore used extensively for estimation of "extracellular fluid" volume, is actually bound to albumin in human serum and therefore is not uniformly distributed throughout the compartment (2). If the concentration of serum albumin is known, its binding effect can be determined by means of a formula (2). However, it is not possible to determine the binding effect of extravascular albumin, since its concentration and distribution cannot be measured.

Gilman, Philips and Koelle suggested the use of another ion, thiosulfate, for estimating "extracellular fluid" volume (3). The present study was undertaken to determine if this anion, like thiocyanate, was bound to any appreciable extent by non-diffusible components of human serum.

Thiocyanate is known to penetrate the red blood cell and values for "thiocyanate space" thus may include the water of the red cell mass (2). To determine if thiosulfate also has this property, measurements of the penetration of this anion into the erythrocyte were also made.

METHODS AND PROCEDURES

The distribution of thiosulfate across cellophane membranes in both the serum and erythrocyte experiments was determined by the same dialysis method previously employed for thiocyanate (2).

1. Experiments with serum and with saline

Twenty mls. of normal human serum were pipetted into cellophane sacs. These were placed in tubes containing 40 mls. of 0.15 molar sodium chloride in which known amounts of thiosulfate were present. Duplicate tubes were dialyzed for 72 hours at 2° C. This low temperature minimizes bacterial action on the sera and has no significant effect on binding, since the binding of chloride and of thiocyanate to albumin is the same at 4° C. as at 25° C. (4, 5).

After dialysis the volume inside and outside was measured and the water content determined by drying to constant weight. The outside thiosulfate concentration was measured in duplicate by the "direct" method of Newman, Gilman, and Philips (6).

Five serum controls were dialyzed separately; 0.0012 mole of thiosulfate was placed inside the sac in three tubes and outside the sac in the remaining two. The outside concentration of "free thiosulfate" in the five tubes must be equal at equilibrium. This condition was satisfied in 72 hours, when outside concentrations were 0.0202, 0.0201, 0.0203, 0.0201, and 0.0199 mole/liter of solution. Thus 72 hours was selected as the terminal point of the distribution experiments on serum.

The range of thiosulfate concentrations in these experiments extends from 2 mgs. % to 320 mgs. % and exceeds the usual range of 10 to 60 mgs. % employed in clearance studies. This latter range would be a suitable one for measurement of "thiosulfate space." Duplicate saline

[1] This study was supported in part by Research Grant H-177 (C) of the National Heart Institute, U. S. Public Health Service.

controls at the same thiosulfate concentration were run to measure any binding of the anion by the cellophane sacs.

From a knowledge of the total water content, the total number of moles of thiosulfate in the system, and outside thiosulfate concentration, the number of moles of thiosulfate bound to non-diffusible components of serum was determined as in the studies with thiocyanate (*2*).

Let Δn be defined in terms of known and experimentally determined values as the difference between the amount of the anion known to be present and the amount which can be recovered in the total water in the system: or

$$\Delta n = [n_0 - n_c] - [g + g'](S_2O_3^=)', \qquad (1)$$

where

n_0 = total number of moles of thiosulfate in the system;
n_c = number of moles of thiosulfate bound to cellophane as determined from saline controls;
$g + g'$ = total water of the system in kilograms, with g referring to the water inside, and g′ to the water outside the sac;
$(S_2O_3^=)$ = concentration in moles per Kg. of water of unbound thiosulfate ion inside the membrane, and
$(S_2O_3^=)'$ = concentration of thiosulfate ion outside the membrane.[2]

Let n_b denote the number of moles of bound thiosulfate in serum. Then the relationship of n_b to Δn can be shown by means of the Donnan equilibrium (*2*) which for the systems as described becomes:

$$\left[\frac{2\Delta n}{g} + 2(S_2O_3^=)' + 0.15\right]\left[\frac{\Delta n - n_b}{g} + (S_2O_3^=)'\right] = [0.15 + 2(S_2O_3^=)'][(S_2O_3^=)']. \qquad (2)$$

The left-hand member of the equation represents the product of the sodium concentration and the thiosulfate concentration inside the sac. The right-hand member is the product of the sodium concentration and the thiosulfate concentration outside the sac.

Dividing equation 2 by $[0.15 + 2(S_2O_3^=)']$ one obtains

$$\left[\frac{\frac{2\Delta n}{g}}{0.15 + 2(S_2O_3^=)'} + 1\right]\left[\frac{\Delta n - n_b}{g} + (S_2O_3^=)'\right] = [(S_2O_3^=)']. \qquad (3)$$

Inspection of the first factor on the left indicates that when Δn approaches zero the value of the first factor on the left approaches 1, and at zero, equation 3 reduces to:

$$\Delta n = n_b = 0. \qquad (4)$$

If there is no binding, the experimentally determined values of Δn would be expected to vary in a chance distribution about zero. If there is binding, this relationship, $\Delta n = n_b$, would not hold and n_b can be calculated from equation *3*.

It has been assumed in this derivation that the sodium ion is not bound appreciably to serum proteins (*4*, *5*).

[2] Primed quantities refer to outside protein-free solution and unprimed quantities to the solution within the sac.

2. Permeability experiments with red blood cells

To determine if thiosulfate penetrates the erythrocyte, dialysis experiments similar to the studies on serum were performed. Fresh defibrinated blood from a healthy donor was centrifuged and washed four times with 0.15 M sodium chloride. The hematocrit after the final washing and centrifugation was 98%.

A preliminary experiment to assure measurement at equilibrium was performed as in the case of the serum experiments. Ten mls. of washed erythrocytes were pipetted into cellophane sacs in a series of tubes numbered 1 to 8. Identical amounts of thiosulfate, 2.43×10^{-4} moles, in 0.15 M sodium chloride were pipetted directly into the tube outside the sacs in tubes 1 and 2, and inside the sacs in tubes 3 and 4. The final volume of the entire tube was made up to 60 mls. with 0.15 M sodium chloride, 20 mls. inside and 40 mls. outside the sac. Tubes 5, 6, 7, and 8 were prepared exactly as the first four but the amount of thiosulfate used was 1.21×10^{-4} moles. This quantity was placed inside the sac in tubes 5 and 6, and outside in tubes 7 and 8. After dialyzing at 2° C. the contents were measured, the outside thiosulfate concentration determined, and well-mixed inside and outside aliquots were dried to constant weight to determine the water content. At 48 hours the thiosulfate concentration $(S_2O_3^=)'$ in tubes 1, 2, 3, and 4 was 0.00407, 0.00407, 0.00408, and 0.00409 mole per Kg. of water. The concentration in tubes 5, 6, 7, and 8 was 0.00181, 0.00183, 0.00155, and 0.00186 mole per Kg. of water, respectively. Thus 48 hours was selected as the terminal point of the erythrocyte penetration experiments.

The penetration of thiosulfate into the erythrocyte can be determined from a knowledge of the total thiosulfate in the system, n_0, from the total water content including the red blood cell water $[g + g']$, and from the thiosulfate concentration outside the sac $(S_2O_3^=)'$, by comparing the quantity $(n_0 - n_c)$ with the quantity represented by $[g + g'](S_2O_3^=)'$ (equation *1*).

In the experiments with red blood cells, no attempt was made to correct for the Donnan equilibrium across the red blood cell membrane.

RESULTS

The results of the experiments are shown in Table I with the saline controls (Part A), the serum dialysis experiments (Part B), and the erythrocyte penetration studies (Part C). The columns are numbered 1 to 8. Column 1 is the tube number. Column 2 is the number of moles of thiosulfate known to be present in each system, n_0. Column 3 is the thiosulfate concentration $(S_2O_3^=)'$, of the outside solution. Column 4 is the concentration inside the cellophane sac $(S_2O_3^=)$, as determined experimentally. Column 5 represents the total water of the system $[g + g']$ and Column 6 the water inside the sac, g. The

TABLE I

Distribution experiments with thiosulfate

	1	2	3	4	5	6	7	8
	Tube no.	Thiosulfate in system (*moles*)	"Outside" thiosulfate concentration (*moles/Kg. H_2O*)	"Inside" thiosulfate concentration (*moles/Kg. H_2O*)	Total H_2O of system *Kg.*	H_2O inside sac *Kg*	Free thiosulfate (*moles*)	Δn (*moles*)
		$n_8 \times 10^4$	$(S_2O_3^=)' \times 10^3$	$(S_2O_3^=) \times 10^3$	$[g+g']$	g	$[g+g'](S_2O_3^=)' \times 10^4$	$\Delta n \times 10^6$
A. Saline Controls*	1	11.98	20.1	19.8	0.0606	0.0195	12.18	−20.0
	2	11.98	20.0	19.8	0.0605	0.0195	12.10	−12.0
	3	9.072	14.7	14.6	0.0612	0.0189	8.90	+17.2
	4	9.072	15.0	14.7	0.0601	0.0190	9.02	+ 5.2
	5	3.080	5.41	5.20	0.0584	0.0201	3.16	− 8.0
	6	3.080	5.34	5.38	0.0579	0.0192	3.12	− 4.0
	7	3.024	4.98	4.86	0.0594	0.0194	2.96	+ 6.4
	8	3.024	4.91	4.88	0.0592	0.0192	2.91	+11.4
	9	0.770	1.35	1.34	0.0588	0.0196	0.790	− 2.0
	10	0.770	1.36	1.38	0.0578	0.0193	0.780	− 1.0
	11	0.320	0.529	0.530	0.0593	0.0204	0.314	+ 0.6
	12	0.320	0.535	0.536	0.0579	0.0197	0.310	+ 1.0
	13	0.0816	0.134	0.146	0.0593	0.0196	0.0794	+ 0.22
	14	0.0816	0.134	0.126	0.0586	0.0196	0.0785	+ 0.31
B. Serum vs. Saline Systems†	15	11.98	21.8		0.0592	0.0255	12.91	−93.0
	16	11.98	20.1		0.0587	0.0236	11.80	+18.0
	17	9.072	15.0		0.0605	0.0224	9.08	− 0.8
	18	9.072	14.6		0.0602	0.0235	8.79	+28.2
	19	3.08	5.42		0.0581	0.0259	3.15	− 7.0
	20	3.08	5.35		0.0573	0.0255	3.07	+ 1.0
	21	3.024	5.18		0.0578	0.0251	2.99	+ 3.4
	22	3.024	5.10		0.0587	0.0249	2.99	+ 3.4
	23	0.770	1.35		0.0582	0.0255	0.786	− 1.6
	24	0.770	1.35		0.0575	0.0249	0.776	− 0.6
	25	0.320	0.527		0.0587	0.0265	0.309	+ 1.1
	26	0.320	0.560		0.0561	0.0243	0.314	+ 0.6
	27	0.0816	0.146		0.0579	0.0240	0.0845	− 0.29
C. Erythrocyte vs. Saline Systems‡	31	2.43	4.07		0.0573	0.0180	2.33	+10
	32	2.43	4.07		0.0563	0.0176	2.29	+14
	33	2.43	4.07		0.0544	0.0174	2.21	+22
	34	2.43	4.09		0.0561	0.0172	2.29	+14
	35	1.21[3]	1.81		0.0572	0.0180	1.03	+18
	36	1.21	1.83		0.0564	0.0182	1.03	+18
	37	1.21	1.55		0.0563	0.0176	0.87	+34
	38	1.21	1.86		0.0567	0.0183	1.05	+16

* For saline controls mean $\Delta n \pm$ S.E. $= (-.33 \pm 1.13) \times 10^{-6}$.

† For serum systems mean $\Delta n \pm$ S.E. $= (-3.94 \pm 9.58) \times 10^{-6}$.

‡ For erythrocyte systems mean $\Delta n \pm$ S.E. $= (18.2 \pm 2.6) \times 10^{-6}$.

seventh column represents the free thiosulfate of the entire system in moles $[g + g'](S_2O_3^=)'$. The eighth column represents the values obtained for Δn. In saline controls, Δn represents thiosulfate bound to cellophane, n_c.

A. Experiments with saline

If there were no appreciable binding to cellophane, the values of Δn in Column 8, Part A, would be expected to fall in a chance distribution about zero, the deviations from zero representing experimental variability. Inspection suggests that the observed values do in fact represent chance deviations from zero. To evaluate this hypothesis, a "t" test was used with the standard error of the mean Δn computed from the variance within duplicate experiments. The mean Δn for saline controls was -0.33×10^{-6}. A difference from zero of this amount or greater could occur by chance with a probability of about 0.8. The observed values are thus consistent with the belief that there is no appreciable binding to cellophane.

B. Experiments with serum

Since no binding to cellophane could be demonstrated for the saline controls, n_c becomes zero and for serum, Column 8 represents $\Delta n = n_0 - [g + g'](S_2O_3^=)'$. As indicated above in equation 4, when $n_b = 0$, $\Delta n = n_b$. Inspection of Column 8 for serum suggests that the values of Δn represent chance deviations from zero, with a mean deviation of -3.94×10^{-6}. The probability of observing a chance difference of this size or greater is about 0.7. Thus there is no appreciable binding to any non-diffusible component of serum and hence not to albumin.

C. Experiments with red blood cells

Since $n_c = 0$, the equation again becomes $\Delta n = n_0 - [g + g'](S_2O_3^=)'$. Table I, Part C, reveals that in all systems $\Delta n > 0$. The observed values of Δn thus indicate that in all tubes some thiosulfate is not accounted for.

DISCUSSION

In the range studied, which extended from about 2 mgs. % to 320 mgs. %, no measurable binding of thiosulfate to non-diffusible components of human serum was noted. Thus, with respect to serum, thiosulfate satisfies the general requirement of "uniform distribution throughout the compartment."

At concentrations of about 30 and 60 mgs. % under the experimental conditions (2° C. over a 48 hr. period) a small amount of thiosulfate in each system was unaccounted for. The results of the experiments in Part C appear to indicate that the concentration of thiosulfate in the red cell is higher than the concentration outside of the red cell, *i.e.*, that there may be binding of thiosulfate to the red cell. There is a discrepancy in that Δn is greater in tubes 35 through 38 inclusive than it is in tubes 31 through 34 inclusive (with the exception of tube 33), although the total thiosulfate in tubes 31–34 is twice that in tubes 35–38. Such a result is contrary to the law of mass action. Finally the possibility remains that the red cells may have destroyed some thiosulfate by their metabolism. The anomalous results just referred to might then be explained if the metabolism of the red cell were depressed in the presence of the higher thiosulfate concentration.

The experiments reported here give no information regarding the rate of penetration of thiosulfate into the red blood cell. If *in vivo* penetration is slow, the effect is the same as that of another route of excretion and hence can easily be corrected for by extrapolation of logarithmic values for serum concentration to zero time and, in this instance, the red blood cell water would not enter into the determined value for thiosulfate space. If *in vivo* red blood cell penetration is as rapid as diffusion into extracellular fluid space, the red blood cell water becomes a functional part of the "extracellular fluid" volume and would be included in the value for thiosulfate space. The total red blood cell water amounts to a volume of about 1,400 to 1,500 mls. when calculated by multiplying the total erythrocyte volume by 0.65, the fraction of water in the erythrocyte (7, 8). For a 70 Kg. man this quantity is approximately 8.5% of the thiocyanate space (uncorrected for binding by albumin—authors' observations).

The concept that the distribution of thiosulfate in the body is predominantly confined to the extracellular spaces depends upon the evidence that the anion is largely excreted by glomerular filtration. Gilman, Philips, and Koelle estab-

lished in the dog a thiosulfate creatinine clearance ratio of unity (3). Extension of these studies to the human kidney demonstrated that thiosulfate is excreted by the glomerulus, since thiosulfate and inulin clearances were identical (6). It is possible to apply clearance data and calculate a volume of distribution for the material studied by means of the formula $V = \frac{C}{S}$, when V represents the volume of distribution, C the clearance and S the slope of the logarithm of the serum concentration with respect to time (9). The application of this formula to the clearance data of the dog by Gilman, Philips, and Koelle revealed volumes of distribution ranging from 21.3% to 24.2% of the body weight (3). Schwartz estimated clearance by means of an infusion technique, and obtained thiosulfate spaces in two humans of 15.7 and 19.5% body weight respectively (10). These figures for thiosulfate space are in fairly close agreement with inulin space measurements (1).

Ideally a substance used for fluid compartment measurements should not be metabolized or the metabolism should be known and correctable. A portion of injected thiosulfate is metabolized and excreted as sulfate (11, 12). The sites of oxidation may be the liver and kidney (11, 13). Evidence obtained in the dog with ligated ureters indicates that thiosulfate is slowly destroyed at a rate proportional to its plasma concentration (3). This value averages only 7% of the renal clearance. Should this logarithmic relationship hold in the human subject, correction for metabolic destruction is possible.

Thiosulfate has a long history of use in clinical medicine, where it has been given extensively by the intravenous route for heavy metal poisoning. The anion is considered non-toxic except for occasional nausea and vomiting (14, 15).

CONCLUSIONS

1. Thiosulfate is not appreciably bound by non-diffusible components of human serum.

2. Thiosulfate penetrates the human erythrocyte.

3. Since it appears to be distributed predominantly in the "extracellular fluid" and is non-toxic, thiosulfate may be useful for estimating the size of the "extracellular fluid" volume.

ACKNOWLEDGMENT

We wish to acknowledge the assistance and cooperation of Dr. I. Herbert Scheinberg for his help in the planning of this study, and Dr. W. E. Reynolds for his advice and assistance in the interpretation of the data.

REFERENCES

1. Levitt, M. F., and Gaudino, M., Measurement of body water compartments. Am. J. Med., 1950, **9**, 208.
2. Scheinberg, I. H., and Kowalski, H. J., The binding of thiocyanate to albumin in normal human serum and defibrinated blood with reference to the determination of "thiocyanate space." J. Clin. Invest., 1950, **29**, 475.
3. Gilman, A., Philips, F. S., and Koelle, E. S., The renal clearance of thiosulfate with observations on its volume distribution. Am. J. Physiol., 1946, **146**, 348.
4. Scatchard, G., Scheinberg, I. H., and Armstrong, S. H., Jr., Physical chemistry of protein solutions. IV. The combination of human serum albumin with chloride ion. J. Am. Chem. Soc., 1950, **72**, 535.
5. Scatchard, G., Scheinberg, I. H., and Armstrong, S. H., Jr., Physical chemistry of protein solutions. V. The combination of human serum albumin with thiocyanate ion. J. Am. Chem. Soc., 1950, **72**, 540.
6. Newman, E. V., Gilman, A., and Philips, F. S., The renal clearance of thiosulfate in man. Bull. Johns Hopkins Hosp., 1946, **79**, 229.
7. Hawk, P. B., Oser, B. L., and Summerson, W. H., Practical Physiological Chemistry. The Blakiston Co., Philadelphia, 1947, Ed. 12, p. 422.
8. Gibson, J. G., II, Peacock, W. C., Seligman, A. M., and Sack, T., Circulating red cell volume measured simultaneously by the radioactive iron and dye methods. J. Clin. Invest., 1946, **25**, 838.
9. Newman, E. V., Bordley, J., III, and Winternitz, J., The interrelationships of glomerular filtration rate (mannitol clearance), extracellular fluid volume, surface area of the body, and plasma concentration of mannitol. Bull. Johns Hopkins Hosp., 1944, **75**, 253.
10. Schwartz, I. L., Measurement of extracellular fluid by means of a constant infusion technique without collection of urine. Am. J. Physiol., 1950, **160**, 526.
11. Elaut, L., Excretion of sodium thiosulphate by the perfused kidney. J. Urol., 1931, **26**, 241.
12. Franklin, J., Genest, J., and Newman, E., The mechanism of excretion of ammonium thiosulfate. Bull. Johns Hopkins Hosp., 1947, **81**, 168.
13. Pirie, N. W., The oxidation of thiosulphate to sulphate by tissue slices *in vitro*. Biochem. J., 1934, **281**, 1063.
14. Litwins, J., Boyd, L. J., and Greenwald, L., The action of sodium thiosulphate on the blood. Exper. Med. & Surgery, 1943, **1**, 252.
15. Goodman, L., and Gilman, A., The Pharmacological Basis of Therapeutics. Macmillan Co., New York, 1941, p. 743.

AMINO ACID STUDIES AND CLINICAL FINDINGS IN NORMAL ADULTS AND RHEUMATOID ARTHRITIS PATIENTS TREATED WITH ACTH[1, 2]

By A. L. BORDEN, E. C. BRODIE, E. B. WALLRAFF, W. P. HOLBROOK, D. F. HILL, C. A. L. STEPHENS, JR., R. B. JOHNSON, AND A. R. KEMMERER

(From the Southwestern Clinic and Research Institute, Inc., and The Department of Nutrition, University of Arizona, Tucson, Arizona)

(Submitted for publication August 30, 1951; accepted February 5, 1952)

Previous publications from this laboratory (1, 2) have reported that rheumatoid arthritis patients who were treated with ACTH (adrenocorticotrophic hormone) and cortisone (17-hydroxy-11-dehydrocorticosterone) exhibited changes in the urinary excretion of certain amino acids. Similar results were found to occur during remission induced by pregnancy (3, 4), and one rheumatoid arthritis patient in remission caused by jaundice showed changes even more striking (5). Significant differences have been reported to exist in plasma concentrations of certain "free" amino acids in untreated rheumatoid arthritis patients and in normal individuals (6). It was considered important, therefore, to determine whether in this disease changes occur in plasma concentrations concomitantly with remission induced by ACTH therapy and also to establish the effect of ACTH on plasma concentrations and urinary excretion of amino acids in normal adults.

Results for six apparent "free" amino acids in the urinary excretion of normal adults and for seven "free" amino acids in plasma of normal individuals treated with ACTH are compared with those from rheumatoid arthritis patients similarly treated.

METHODS

The normal control group consisted of four male and four female university students. Immediately before the experiment each student was subjected to a complete physical examination and absence of any disease or obvious metabolic disorder was established. The students were not hospitalized but were kept on a closely supervised diet of adequate caloric and vitamin C intake and one which supplied one gram of protein per kilogram of body weight. The source of protein was the same for all subjects and to that extent the amino acid intake was controlled. Following a three-day control period on the diet, 40 mg. of ACTH were administered intramuscularly in three divided doses each day for seven days. Thirty-five patients were chosen on the basis of an unquestionable diagnosis of rheumatoid arthritis and had been under treatment by the investigators for periods ranging from six months to 15 years. They were hospitalized for observation and study throughout the experiment and were maintained on the same controlled diet as that used for the normal adult group. ACTH was administered intramuscularly in variable dosage of from 40 to 60 mg. daily except for one patient who received a much larger dose. It was determined conclusively that the size of the dose had no measurable effect on the plasma levels of the amino acids assayed. In an effort to accelerate or prolong the favorable effects of ACTH therapy some patients included in this study received supplementary medication. During the control period and/or through the treatment period various agents such as ATP (adenosine triphosphate) ascorbic acid, thyroid, testosterone, aspirin, and amino acid feedings were used. None of these supplements altered the significant amino acid increases induced by ACTH.

Twenty-four hour urine specimens were collected on two days prior to the administration of ACTH. During treatment, specimens were collected on alternate days. Aliquots of the samples were stored at − 15° C.

By using the method of Hier and Bergeim (7), tungstic acid filtrates were prepared from fasting blood samples obtained before and on alternate days of treatment. Filtrates were stored in the frozen state until time of assay. A modification of the microbiological technic of Henderson and Snell (8) was used for the method of assay. *Lactobacillus mesenteroides* and *Streptococcus fecalis R* were the test organisms employed. All specimens from one individual were analyzed in one series to eliminate errors due to variations in organism response. The samples were measured in duplicate and at three levels of dilution. In all other details the procedures as previously described (7, 8) were followed. In an earlier publication (1) we established that ACTH has no inhibi-

[1] This work was supported in part by grants from the United States Public Health Service and the Fair Foundation. ACTH was provided by the Armour Laboratories.

[2] A preliminary report of this material was presented before the Federation of American Societies for Experimental Biology, Cleveland, Ohio, April 1951.

tory or stimulatory effect on the above mentioned microorganisms. In the same publication it was proven that supplementary medication does not alter the pattern of urinary excretion of amino acids induced by treatment with ACTH. Therefore, patients receiving ACTH with or without supplementary medication were compared to normal adults receiving only ACTH. The effect of supplementary medication on amino acid values in the plasma is discussed later. The results shown in this paper were statistically analyzed by the Multiple Variance Method (9) and the Student's *t* Test (10).

RESULTS

Clinical

The clinical findings in the group of rheumatoid arthritis patients were the same as those reported in one of our earlier publications (1).

Interestingly enough the group of normal adults did not experience the findings manifested by the patients with the disease. There was no increase in appetite, no indication of insomnia, no increase in energy, nor any measurable change in mental alertness. Weight was maintained at a relatively constant level throughout the experiment. There were no indications of toxicity. In short, upon careful examination it was impossible to detect any difference in the way the normal adults reacted before beginning the medication and during its administration.

Chemical

Urine: In Table I the comparison is made between the urinary excretion of six apparent "free" amino acids in rheumatoid arthritis patients and normal adults treated with ACTH. The response to the medication gives a "curve" type reaction in which excretion values rise to a "peak" or maximum level and, while medication is still being administered, gradually level off. All treatment values, however, are well above control levels established before institution of therapy. Previous work (1, 2) reported that in rheumatoid arthritis patients treated with ACTH, the average and maximum 24-hour excretion of histidine, lysine, threonine, and tyrosine showed a 1% significant increase. In the normal adults the urinary excretion of none of the above mentioned amino acids was significantly increased with the exception of threonine for which there was a significant 5% increase at the maximum level only. Phenylalanine and tyrosine were increased at the 1% significant level but only at the time of maximum excretion. The degree of response to ACTH was significantly less in the urinary excretion of normal adults than in patients with rheumatoid arthritis. The single exception was phenylalanine where the reverse was true at the maximum level. In neither group was the excretion of arginine altered. Tyrosine

TABLE I

Average and maximum 24-hour urinary excretion of six apparent "free" amino acids in rheumatoid arthritis patients and in normal adults treated with ACTH

Amino acid	Type subject	No.	Before treatment (mean)	During treatment (average mean)	During treatment (maximum)
			mg./24 hr.	*mg./24 hr.*	*mg./24 hr.*
Arginine	Arthritis	31	5.8± 1.30	6.1± 1.0	8.3± 1.28
	Normal	8	8.3± 0.74	10.0± 0.84	11.3± 0.94
Histidine	Arthritis	31	58.0± 6.91	126.0±12.42*†	169.0±16.85*†
	Normal	8	137.0±14.14	174.0±15.57	195.0±16.65‡
Lysine	Arthritis	31	21.4± 2.24	46.7± 5.78*†	67.2± 8.48*†
	Normal	8	23.4± 7.00	25.2± 8.19	32.0± 8.52
Phenylalanine	Arthritis	12	7.3± 0.95	11.4± 1.66	15.5± 3.07‡
	Normal	8	9.8± 0.76	12.3± 0.32‡	13.3± 0.32*
Threonine	Arthritis	31	16.6± 2.98	43.7± 7.57*†	65.2±12.38*†
	Normal	8	17.2± 1.13	23.2± 2.52‡	26.3± 2.29‡
Tyrosine	Arthritis	20	19.7± 2.23	32.3± 3.17*†	43.2± 4.06*†
	Normal	8	12.3± 0.89	14.7± 1.26	17.4± 1.14‡

* Significant at the 1% level (Analysis of Variance).
† Significantly different from normal at 1% level (*t* Test).
‡ Significant at the 5% level (Analysis of Variance).

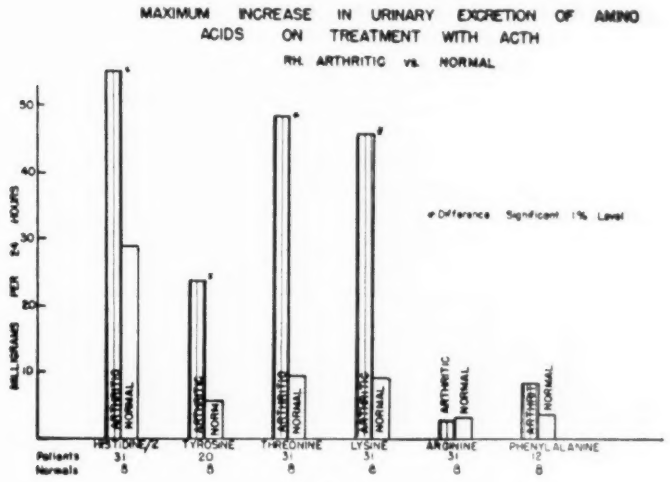


FIG. 1

and histidine values before treatment are significantly different for normal individuals and patients with the disease. The maximum increases in urinary excretion of amino acids in the two treated groups are shown in Figure 1.

Plasma: The same "curve" type response which was noted in the urinary excretions occurred also in the plasma estimations. As seen in Table II, at the maximum plasma concentrations observed during treatment, the group of rheumatoid arthritis patients showed a significant increase for each of the seven amino acids. The average values during treatment were increased significantly for arginine, lysine, and threonine.

TABLE II

Average and maximum plasma concentrations for seven apparent "free" amino acids in rheumatoid arthritis patients and normal adults treated with ACTH

Amino acid	Type subject	No.	Before treatment (mean)	During treatment (average mean)	During treatment (maximum)
			gamma/ml.	*gamma/ml.*	*gamma/ml.*
Arginine	Arthritis	35	13.8±1.33	17.5±1.71†‡	19.4±1.98*†
	Normal	8	15.0±0.90	15.5±0.84	17.2±1.04
Histidine	Arthritis	34	11.0±0.71	11.9±0.80	13.3±0.99‡
	Normal	8	14.2±0.86	12.8±0.96	14.0±1.02
Lysine	Arthritis	35	27.9±2.01	34.5±2.31*†	38.7±2.69*†
	Normal	8	26.1±1.96	24.1±1.42	26.9±1.80
Phenylalanine	Arthritis	20	12.1±0.52	12.9±0.54	15.1±0.86*†
	Normal	8	10.9±0.61	11.3±0.40	12.1±0.48
Proline	Arthritis	13	21.6±1.77	24.9±1.93	29.2±2.39*
	Normal	8	29.4±2.17	30.4±2.74	33.4±2.40
Threonine	Arthritis	35	12.9±1.2	17.9±0.97*†	20.4±1.29*†
	Normal	8	13.4±1.15	13.8±0.42	15.4±0.41
Tyrosine	Arthritis	18	13.9±0.96	14.6±1.12†	17.2±1.37†‡
	Normal	8	9.8±0.79	9.6±0.71	10.4±0.87

* Significant at the 1% level (Analysis of Variance).
† Significantly different from normal at 1% level (*t* Test).
‡ Significant at the 5% level (Analysis of Variance).

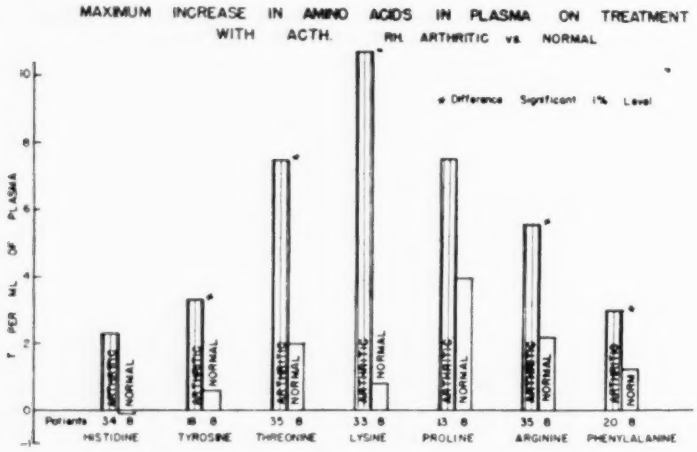


FIG. 2

While the plasma values for the group of normal individuals showed the same general trend as the rheumatoid arthritis patients, the changes observed, both at the time of maximum increase and at the average level, were not significantly increased over the control values obtained before treatment.

The behavior of the treated rheumatoid arthritis group as compared with the group of normal adults was significantly different in several instances. At the maximum plasma response the difference in the way the groups reacted was highly significant for arginine, lysine, phenylalanine, threonine, and tyrosine. The maximum value response was not significantly different for histidine or proline. The average treatment plasma values for the two groups were significantly different at the 1% level for arginine, lysine, threonine, and tyrosine and not significantly altered for histidine, phenylalanine, or proline. The maximum increases in plasma amino acids for the two groups treated with ACTH are shown in Figure 2.

Our own investigations, as well as the work of others (11), have established that plasma values for the amino acids are relatively constant in an individual. It seems, therefore, that these significant differences due to therapy with ACTH are worthy of consideration. The small but significant plasma increases, when considered in terms of total circulating blood volume, indicate an appreciable change.

By means of an analysis with the Multiple Variance Test (9), the results from patients with rheumatoid arthritis treated simultaneously with supplementary medication and ACTH were compared with those from the group whose treatment was uncomplicated. It was conclusively proven that those plasma amino acid concentrations which were significantly increased by ACTH therapy were not significantly affected by supplementary medication.

DISCUSSION

We have no adequate explanation for the changes in amino acid concentrations in plasma levels and in urinary excretions. A number of possibilities have been considered to explain the mechanism at work but we have as yet no actual proof to substantiate them. All indications are that we are measuring changes in intrinsic protein metabolism. Although the mechanism of amino aciduria is not understood, a lowered renal threshold does not appear to be involved. Tolerance tests with histidine and threonine, now in progress in this laboratory, have shown that even when an oral dose of from 2 to 10 grams is employed, only 2% to 5% of the amino acid is recovered in the urinary excretion (12).

It is interesting to note that the urinary excretion of arginine is not increased in either group while the plasma levels for arginine in the rheumatoid arthritis group are significantly increased.

Of further interest is the fact that the value for histidine, the amino acid excreted in such large amounts in the urine in remissions induced by pregnancy, jaundice, cortisone, or ACTH, is not altered to a large degree in the plasma.

Because the data here presented show such significant differences between the reactions of the two groups to ACTH therapy, additional evidence seems provided of a correlation between amino acid metabolism and the remission factor in rheumatoid arthritis.

SUMMARY

In a group of normal individuals treated with ACTH, microbiological assays for six "free" amino acids were determined on 24-hour urine samples, and for seven amino acids on plasma filtrates. The values have been compared with those found in rheumatoid arthritis patients similarly treated.

Unlike the clinical findings in patients with rheumatoid arthritis, the treated normal adults manifested no improvement in appetite, increased energy, mental alertness, or sense of general well-being.

Urinary excretion and plasma values for the rheumatoid arthritis group exhibited appreciable increases during the treatment with ACTH. The most significant changes occurred for histidine, lysine, threonine, and tyrosine in the urine, and for arginine, lysine, and threonine in the plasma.

The results for the normal adults followed the same general trend in response but to a strikingly less degree. In the urinary findings only phenylalanine and tyrosine were significantly increased during treatment and then only at the time of maximum excretion. None of the plasma amino acids assayed was significantly increased at any time during the treatment.

A highly significant difference in amino acid response of the two groups occurred for histidine, lysine, threonine, and tyrosine in the urine, and for arginine, lysine, threonine, and tyrosine in the plasma. It is interesting to note that the histidine changes are limited to the urinary excretion findings while the arginine changes are peculiar to the plasma.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. M. G. Vavich for his criticisms and suggestions and to Mr. Joel E. Fletcher for his valuable help with the statistical analyses and charts. Further appreciation is extended to the group of students for their excellent cooperation in making this study possible.

REFERENCES

1. Stephens, C. A. L., Jr., Wallraff, E. B., Borden, A. L., Brodie, E. C., Holbrook, W. P., Hill, D. F., Kent, L. J., and Kemmerer, A. R., Apparent free histidine plasma and urine values in rheumatoid arthritis treated with cortisone and ACTH. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 275.
2. Brodie, E. C., Wallraff, E. B., Borden, A. L., Holbrook, W. P., Stephens, C. A. L., Jr., Hill, D. F., Kent, L. J., and Kemmerer, A. R., Urinary excretion of certain amino acids during ACTH and cortisone treatment of rheumatoid arthritis. Proc. Soc. Exper. Biol. & Med., 1950, **75**, 285.
3. Unpublished data.
4. Wallraff, E. B., Brodie, E. C., and Borden, A. L., Urinary excretion of amino acids in pregnancy. J. Clin. Invest., 1950, **29**, 1542.
5. Borden, A. L., Wallraff, E. B., Brodie, E. C., Holbrook, W. P., Hill, D. F., Stephens, C. A. L., Jr., Johnson, R. B., and Kemmerer, A. R., Effect of adrenocorticotrophic hormone on amino acid content of blood and urine in normal individuals and patients with rheumatoid arthritis. Federation Proc., 1951, **10**, 376.
6. Borden, A. L., Wallraff, E. B., Brodie, E. C., Holbrook, W. P., Hill, D. F., Stephens, C. A. L., Jr., Kent, L. J., and Kemmerer, A. R., Plasma levels of free amino acids in normal subjects compared with patients with rheumatoid arthritis. Proc. Soc. Exper. Biol. & Med., 1950, **75**, 28.
7. Hier, S. W., and Bergeim, O., Microbiological determination of free leucine, isoleucine, valine, and threonine in dog plasma. J. Biol. Chem., 1945, **161**, 717.
8. Henderson, L. M., and Snell, E. E., A uniform medium for determination of amino acids with various microorganisms. J. Biol. Chem., 1948, **172**, 15.
9. Snedecor, G. W., Statistical Methods Applied to Experiments in Agriculture and Biology. Collegiate Press, Inc., Ames, Iowa, 1946, Ed. 4, Section 10.4.
10. Snedecor, G. W., *Ibid.*, Section 3.8.
11. Kirsner, J. B., Sheffner, A. L., and Palmer, W. L., Studies on amino acid excretion in man. III. Amino acid levels in plasma and urine of normal men fed diets of varying protein content. J. Clin. Invest., 1949, **28**, 716.
12. Unpublished data.

THE LUNG VOLUME AND ITS SUBDIVISIONS IN NORMAL BOYS 10–17 YEARS OF AGE [1]

By MINERVA MORSE, FREDERIC W. SCHLUTZ,[2] AND DONALD E. CASSELS

(From the Department of Pediatrics, University of Chicago Clinics, Chicago, Ill.)

(Submitted for publication November 14, 1951; accepted February 5, 1952)

Data on the lung volume and its subdivisions were collected as part of a study of the functional fitness of normal boys 10–17 years of age (1). Since, in the case of children of all ages, so little is known concerning the total lung capacity and its subdivisions other than the vital capacity, the data are of value in helping to establish the range of variation in the normal child and to provide one basis for the evaluation of pulmonary function in disease.

Because the absolute values obtained have no meaning for children varying so greatly in size, except as they can be referred to some standard of reference, such as height, weight, and other measurements of body size, the results were analyzed by statistical methods in order to determine the relation of the lung volume and its subdivisions to various physical characteristics of the child. These relationships were then expressed in the form of regression equations which can be used to predict the lung volume and its subdivisions for a given individual and, by means of the standard error of estimate, can give a measure of the amount of variation that may be expected in the normal child.

Of the various subdivisions of the lung volume the vital capacity has been studied extensively in children and many formulae have been developed by which the "normal" vital capacity can be estimated from such measurements as standing or sitting height, weight, chest girth, surface area and combinations of these variables, some of which have included age. Metheny has given an excellent resumé of the literature on the subject (2, 3). A recent study by Turner and McLean (4) found that the mean inspiratory reserve constituted 76.0 per cent of the vital capacity and the expiratory reserve 24.0 per cent in children six to 14 years of age while in the recumbent position. Comparatively little is known about the total lung capacity and the residual volume. Robinson (5) studied the subdivisions of the lung volume for the whole age range from five to 91 years. His results were reported as absolute values and in terms of units of weight, height, and surface for various age groups, three of which fall within the age range of this study. His study was made before Lassen, Cournand, and Richards (6) suggested a modification of the closed-circuit method of Christie (7) for the determination of functional residual capacity, which would allow for an "oxygen storage" effect by including in the calculations nitrogen concentrations in alveolar samples taken at the beginning and end of the test.

DEFINITION AND NOMENCLATURE

The nomenclature recommended by a group of members of the American Physiological Society (8) has been used rather than other terms which have commonly appeared in the literature. According to this nomenclature, the inspiratory capacity is the maximum volume of air that can be taken into the lungs above the normal expiratory level, whereas the functional residual capacity is the volume of air left in the lungs after the normal expiration. The two together make up the total lung capacity. The expiratory reserve volume is the maximum volume of air that can be voluntarily expelled from the lungs after a normal expiration and the residual volume is the volume of air left in the lungs after the reserve volume has been expelled. The vital capacity is taken as the sum of the inspiratory capacity and the expiratory reserve volume. Ordinarily the latter determination followed immediately after the former. All terms used here refer to the resting, recumbent position.

METHODS

All measurements of the lung volume and its subdivisions were made in the recumbent position with one pillow under the head. The apparatus used for the lung volume determinations was a Sanborn closed-circuit wet spirometer with a kymographic drum and with the soda lime tube placed outside of the spirometer to reduce the dead space of the apparatus as recommended by Christie (7). Inspiratory capacity and expiratory reserve volume were calculated from the calibrated spirometric tracing after a line representing the normal expiratory level had been

[1] This work was conducted under a grant from the Douglas Smith Foundation at the University of Chicago.

[2] Deceased March 8, 1944.

drawn. The maximum of three or four efforts was used. Volumes were corrected to 37° C., saturated with water vapor (BTPS).

Following the recommendation of Hurtado and Boller (9), the residual volume was determined directly rather than the functional residual capacity.[3] In other respects the method for the determination of the residual volume followed the modification of the closed-circuit method of Christie (7) which was suggested by Lassen, Cournand, and Richards (6). Unfortunately the study was begun before the preferable open-circuit method was described by Darling, Cournand, and Richards (15). At the end of a maximum forced expiration following a normal expiration (alveolar sample 1), the subject was connected to the spirometer containing a known amount of oxygen. After seven minutes of breathing through the closed circuit the test was terminated by another forced expiration (alveolar sample 2). The alveolar samples and samples taken from the spirometer were analyzed for nitrogen in a Haldane apparatus provided with a burette which was graduated between 5 and 10 ml. Residual volumes were calculated from the equation:

$$VR = \frac{F'S_{N_2} \cdot (V'S+VD) - 0.791\,VD - 0.004\,VS - VE_{N_2}}{FA_{N_2} - F'A_{N_2}} \times \frac{310}{273+AT},$$

VR = residual volume at body temperature and ambient pressure, saturated with water vapor (BTPS).

VD = dead space of apparatus, 2,260 ml. in this instance, as determined by the method of Christie (7). This value was maintained constant by keeping the spirometer filled with water up to a fixed point on the spirometer wall. By repeated rinsings the dead space was filled with room air before the test began.

VS = initial volume of the spirometer at ambient temperature and pressure (ATPS). The spirometer was filled with tank oxygen which contained 0.4 per cent nitrogen as an impurity.

V'S = final spirometer level (ATPS) if the final forced expiration was complete = initial volume of spirometer minus oxygen consumed during the test.

VE_{N_2} = volume of nitrogen excreted from the blood in seven minutes. This was calculated from a nomogram, based upon the data of Campbell and Hill (16), which related nitrogen excretion to rate of oxygen consumption and to spirometer nitrogen concentration.[4]

$F'S_{N_2}$ = concentration of nitrogen in the spirometer at the end of the test.

FA_{N_2} and $F'A_{N_2}$ = concentrations of nitrogen in the alveolar samples taken before and after the test.

AT = ambient temperature.

0.791 = fractional nitrogen concentration in room air.

0.004 = fractional nitrogen concentration in tank oxygen.

$\frac{310}{273+AT}$ = factor for converting volumes obtained at ambient temperature to 37°C.

[3] The residual volume was determined directly, rather than the functional residual volume, in the hopes of reducing the error introduced in the calculation of the final spirometer level caused by irregular breathing. In many cases, however, the records showed poor agreement between the expiratory effort at the beginning and end of the test. Before calculating the residual volume each chart was studied carefully. The accuracy of calculation of oxygen consumption during the test was evaluated by comparing the rates of oxygen consumption in duplicate tests with each other and with that of a preliminary test made without forced expirations. The expiratory efforts at the beginning and end of the test were compared with each other and with the records of reserve air made during the preceding determination of vital capacity. Many inconsistencies were found. In each case a choice was made, whether to base calculations *1*) on the final spirometer level measured after maximum expiration, *2*) on a value calculated by subtracting oxygen consumption during the test from the spirometer level at the time the test began, or *3*) on an average obtained by the two methods. Of 180 single determinations, 52 per cent were based upon method *3*, 32 per cent upon method *2*, and only 16 per cent upon method *1*. In case the record showed that the initial forced expiration was incomplete as compared to previous measurements, correction was made by reducing the calculated residual volume by the appropriate amount.

Measurements of residual volume were made on 94 individuals. In 85 of the cases it was possible to complete duplicate determinations. In 11 cases one of the duplicate values was discarded, usually because breathing was irregular and the expiratory effort inconsistent. The experimental error in the remaining 74 cases of duplicate determinations was calculated by the method described by Mainland (10) and used in a similar way by Herrald and McMichael (11), McMichael (12), Meneely and Kaltreider (13), and Gilson and Hugh-Jones (14). The results were as follows:

Sum of absolute differences	5,902 ml.
Mean difference (5,902/74)	79.8 ml.
Mean difference from mean of pairs	39.9 ml.
Sum of squares of absolute differences	696,802 ml.[2]
Variance of absolute differences, 696,802/74	9,406 ml.[2]
Standard deviation, $\sqrt{9{,}406}$	±97.0 ml.
S.D. of differences from the mean, or, S.E. of a single determination, $97.0/\sqrt{2}$	±68.6 ml.
S.E. of the mean of a pair of duplicates, $68.6/\sqrt{2}$	±48.5 ml.[2]

For a mean residual volume of 698 ml., this represents a possible percentage error of ± 19.6 per cent for a single determination, or ± 13.8 per cent for the mean of a pair of duplicates, in 95 per cent of the cases.

MATERIAL

The subjects of the study were 124 normal healthy boys between the ages of 10 and 17 years,[5] who participated in

[4] Kindly given to the authors by Dr. Sid Robinson.

[5] We are indebted to the University of Chicago Settlement House, the Valentine Boy's Club, the Hyde Park Neighborhood House and friends for their kindness and interest in sending these boys to the laboratory for the tests.

TABLE I

Correlation coefficients which show the relation between the lung volume and its subdivisions and certain physical characteristics

Independent variable	Total lung capacity	Inspiratory capacity	Functional residual capacity	Expiratory reserve volume	Residual volume	Vital capacity
Age	.754	.723	.690	.638	.615	.736
Height	.932	.924	.793	.772	.679	.933
(Height)^3	.940	.931	.799	.778	.684	.940
Actual weight	.820	.843	.683	.658	.608	.841
Normal weight	.917	.905	.797	.770	.683	.917
Surface area	.915	.915	.772	.730	.684	.915
Chest girth	.862	.845	.770	.747	.661	.863

the other tests for physical fitness which were described earlier (1). They came to the laboratory in the early morning and had rested one-half hour in the recumbent position before measurements of the lung volume and its subdivisions began. Total lung capacity and residual volume determinations were made in only 94 of the boys.

Certain physical characteristics of each boy were determined by methods described by McCloy (17), after all the tests in the recumbent position were completed. These included measurements of height, weight, chest circumference at the level of the xiphoid cartilage, hip (biiliac) width, knee width when flexed at a right angle, and skin and subcutaneous fat thicknesses measured by calipers over the chest, abdomen, back and hips (supra-iliac). Chest circumference was corrected for the presence or absence of subcutaneous fat. From such data the "normal weight," the "index of build," and the "index of fat," of each boy were calculated by the methods of McCloy (18). Surface area was estimated from height and weight by means of a nomogram (19) based on the formula of DuBois and DuBois (20).

RESULTS

As a basis for selecting the best physical characteristics to which individual lung volumes and corresponding subdivisions could be referred, correlation coefficients between the lung volume and its subdivisions and certain physical characteristics were calculated (Table I). The results show that with few exceptions standing height serves somewhat better as a standard of reference than the more complicated measurements of surface or normal weight. In the case of residual volume and functional residual capacity slight preference might be given to normal weight. Age, actual weight and chest girth serve poorly as standards of reference. The higher correlations of the lung volume and its subdivisions with (height)^3 as compared to those with the first power of height are to be expected in view of the three-dimensional character of the lung volume. On the basis of these results standing height was selected as the standard to which to refer the lung volumes found in this study. The mean values of the lung volume and its subdivisions which were found for these height groups are summarized in Table II.

TABLE II

The lung volume and its subdivisions, expressed as mean values and their standard deviations for successive height groups

No. of subjects	Height range	Total lung capacity	Subdivisions of the lung volume				
			Inspiratory capacity	Functional residual capacity	Residual volume	Expiratory reserve volume	Vital capacity
	cm.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
8 (5)*	137.5–142.4	2,738±122	1,654±118	1,020±173	599±132	445±66	2,099±129
19 (12)	142.5–147.4	2,743±280	1,776±182	1,008±190	492±155	525±104	2,301±219
13 (11)	147.5–152.4	3,124±352	2,008±277	1,107±164	581±126	519±106	2,527±331
20 (14)	152.5–157.4	3,471±446	2,207±296	1,279±292	675±201	623±134	2,830±369
12 (8)	157.5–162.4	3,776±491	2,438±334	1,371±324	756±257	626±118	3,064±362
12 (11)	162.5–167.4	4,396±460	2,774±299	1,616±290	880±229	725±149	3,498±361
16 (13)	167.5–172.4	4,594±306	3,033±351	1,585±238	818±172	794±153	3,826±377
13 (11)	172.5–177.4	5,151±475	3,208±265	1,893±377	1,048±273	830±135	4,039±335
6 (5)	177.5–182.4	5,640±529	3,612±434	2,036±236	1,042±137	933±177	4,545±447
4 (4)	182.5–187.4	6,229±436	4,116±316	2,113±325	1,132±300	981± 78	5,097±246
1	190.0		4,290			1,255	5,545

* Quantities in parentheses refer to the number of subjects in whom the residual volume was determined and the functional residual and total lung capacities calculated.

A study of the mean volumes in relation to the corresponding mean heights showed deviations from a linear trend which suggested that the trend with increasing height might best be represented by a curvilinear function. "F" tests for non-linearity [6] proved to be significant in the cases of the total, inspiratory and vital capacities, but gave no support for a non-linear relation to height in the case of the other subdivisions. The amount of individual variation is relatively greater in the case of the reserve, residual and functional residual volumes and therefore non-linearity of trend would be more difficult to prove. Since the correlations of all the subdivisions with $(\text{height})^3$ are higher than with height alone, and since the volumes under consideration are three-dimensional, we assumed that all of the volumes are better represented as a function of $(\text{height})^3$ and continued the analysis of the effect of other factors upon the lung volume and its subdivisions on that basis.

The high correlations of the lung volume and its subdivisions with the various physical characteristics of the growing boy are no doubt due to the fact that all of these measurements are linked with growth and that an increase in one measurement is more or less associated with similar increases in all of the others. To determine to what extent other factors act independently of $(\text{height})^3$ to determine the lung volume of an individual, partial correlation coefficients between the lung volume and its subdivisions and various physical characteristics were calculated, with $(\text{height})^3$ held

[6] The F test for non-linearity is described by Goulden (21). It depends upon an analysis of variance after the data are set up in the form of a correlation table. The total sum of squares of deviations from the mean is split into two portions, one representing deviations of the means of the various arrays from the regression line, and the other representing differences between values within the arrays. The F value represents the ratio between the variances due to these two factors. This is compared with the F values which correspond to probabilities of 0.01 and .05 that the results are due to chance sampling.

TABLE III

Partial correlation coefficients which relate the lung volume and its subdivisions to some physical characteristics of the subjects

Variable	Variables held constant: $(\text{height})^3$	$(\text{height})^3$ and index of fat	$(\text{height})^3$ and chest girth	$(\text{height})^3$	$(\text{height})^3$ and index of fat	$(\text{height})^3$ and chest girth
	Inspiratory capacity and			Total lung capacity and		
Age	.021		−.012	.158		.026
Chest girth	.302	.296		.431	.491	
Actual weight	.239			.196		
Normal weight	.263			.355		
Index of build	.268	.264		.374	.462	
Index of fat			−.048			−.268
	Expiratory reserve volume and			Vital capacity and		
Age	.095		−.004	.051		−.102
Chest girth	.268	.390		.378	.417	
Actual weight	−.030			.198		
Normal weight	.185			.308		
Index of build	.140	.289		.293	.349	
Index of fat			−.350			−.194
	Residual volume and			Functional residual volume and		
Age	.197		.134	.205		.115
Chest girth	.229	.275		.320	.418	
Actual weight	.096			.053		
Normal weight	.178			.246		
Index of build	.249	.325		.300	.418	
Index of fat			−.174			−.321

statistically constant. Correlations with (height)^3, with the various physical characteristics held constant, one or two at a time, were also calculated. In addition to the physical characteristics previously mentioned, all of which include the element of growth, two other characteristics, the index of build, and the index of fat, which are quite independent of the growth factor, were included in the statistical analysis. The results, summarized in Table III, allow the following deductions to be made:

1. With (height)^3 held constant, correlations of the lung volume and all of its subdivisions with index of build or chest girth are high enough to be significant and are positive in sign. Thus the stocky boy with a relatively large thoracic cross-section tends to have a greater lung volume than the slender, narrow-chested boy of the same height.

2. With (height)^3 and chest girth held constant, the correlation of the lung capacity with the index of fat is small but it is significant and is negative in sign. With (height)^3 and index of fat held constant, the correlation of the lung capacity with the index of build or with chest girth is increased over its value with (height)^3 alone constant. The same relationships hold for all of the subdivisions of the lung volume except the inspiratory capacity. The latter shows no significant correlation with the index of fat. The highest correlations with the index of fat pertain to the expiratory reserve volume and to the functional residual volume which contains it. This agrees with the finding of Aslett, Hart and McMichael (*22*) in adults, that an obese group had a substantially decreased relative expiratory reserve and a decreased functional residual capacity as compared with a non-obese group. Thus it appears that the presence of subcutaneous fat reduces the capacity for forced expiration, chiefly by reducing the resting lung volume, but it does not limit the inspiratory capacity.

3. With (height)^3 held constant, the correlations of the lung volume and its subdivisions with normal weight are higher than those with actual weight. For a given height, a greater actual weight reflects a more stocky build, more subcutaneous fat or both. Since the former tends to increase the lung volume while the latter reduces it, poor correlations with actual weight could be expected.

4. The residual volume and functional residual capacity show a significant correlation with age when (height)^3 is held constant, but the value of the correlation coefficient is reduced when chest girth is held constant with height. The correlation coefficient between total lung capacity and age, (height)^3 held constant, is almost high enough to be considered significant, but is reduced almost to zero when chest girth is also held constant. Correlation coefficients between age and the other subdivisions of the lung volume are low and not significant, whether (height)^3, or (height)^3 and chest girth are held constant. The question of the effect of age upon the subdivisions of the lung volume will be discussed in greater detail later in this report.

By the method of least squares, regression equations were developed to express the relations between the lung volume and its subdivisions and the various standards of reference (Table IV). These may be used to predict the lung volume of boys between 10 and 17 years of age. The choice of which standard of reference to use will depend upon the availability of information on each physical characteristic and the accuracy of prediction gained by its use. Usually those physical characteristics which give the smallest error of prediction would be chosen as the appropriate standards, but for many purposes the slight gain in accuracy is overbalanced by practical considerations. Corresponding equations based on (height)^3 and index of build or normal weight were not included in the table because it was found that they yielded no closer prediction than (height)^3 and chest girth. Chest girth represents a direct measurement, whereas index of build and normal weight are calculated values which are based upon a number of body measurements, including chest girth.

Table IV includes simple linear equations based on height, for two height groups, 140 to 160 cm., and 160 to 190 cm. In this case a non-linear relation is expressed quite accurately by a series of linear equations covering smaller ranges of the independent variable. Linear equations offer the practical advantage of ease of calculation and for many purposes they are sufficiently accurate.

The relation of the subdivisions of the lung volume to the cube of the standing height represents one possible curvilinear function which has theorectical significance in that the lung volumes are three-dimensional. Another type of curvilinear relationship, represented by the equation $\log y = a +$

TABLE IV

Regression equations which can be used to predict the lung volume and its subdivisions

The relative accuracy of prediction and the normal range of variation can be estimated from the standard error of estimate since, theoretically, 95 per cent of the values for normal individuals of given physical characteristics will fall within ±2 standard errors from the value predicted from those characteristics by the use of the appropriate equation.

Physical characteristics used as standards of reference	Regression equation*	Standard error of estimate
	Total lung capacity	
Height (140–160 cm.)	$Y = 56.1\ H - 5{,}272$	376
Height (160–190 cm.)	$Y = 99.2\ H - 12{,}120$	369
Height and actual weight	$Y = 70.7\ H + 11.4\ AW - 7{,}930$	378
$(Height)^3$	$Y = 0.001035\ H^3 - 367$	363
$(Height)^3$ and actual weight	$Y = 0.000925\ H^3 + 10.2\ AW - 412$	359
$(Height)^3$ and chest girth	$Y = 0.000796\ H^3 + 37.5\ CG - 2{,}154$	328
$(Height)^3$, chest girth, and index of fat	$Y = 0.000740\ H^3 + 46.3\ CG - 284\ IF - 2{,}273$	316
	Inspiratory capacity	
Height (140–160 cm.)	$Y = 40.3\ H - 4{,}034$	248
Height (160–190 cm.)	$Y = 64.0\ H - 7{,}842$	291
Height and actual weight	$Y = 43.7\ H + 10.2\ AW - 4{,}960$	265
$(Height)^3$	$Y = 0.000676\ H^3 - 297$	265
$(Height)^3$ and actual weight	$Y = 0.000570\ H^3 + 9.6\ AW - 320$	253
$(Height)^3$ and chest girth	$Y = 0.000549\ H^3 + 19.4\ CG - 1{,}202$	248
	Functional residual capacity	
Height (140–160 cm.)	$Y = 19.7\ H - 1{,}811$	239
Height (160–190 cm.)	$Y = 32.9\ H - 3{,}905$	288
Height and actual weight	$Y = 25.6\ H + 2.22\ AW - 2{,}780$	261
$(Height)^3$	$Y = 0.000354\ H^3 - 50$	258
$(Height)^3$ and chest girth	$Y = 0.000227\ H^3 + 19.8\ CG - 993$	244
$(Height)^3$, chest girth, and index of fat	$Y = 0.000177\ H^3 + 27.6\ CG - 253\ IF - 1{,}099$	231
	Vital capacity	
Height (140–160 cm.)	$Y = 49.2\ H - 4{,}819$	294
Height (160–190 cm.)	$Y = 79.9\ H - 9{,}750$	316
Height and actual weight	$Y = 56.2\ H + 9.7\ AW - 6{,}265$	308
$(Height)^3$	$Y = 0.000831\ H^3 - 266$	297
$(Height)^3$ and actual weight	$Y = 0.000731\ H^3 + 9.1\ AW - 294$	291
$(Height)^3$ and chest girth	$Y = 0.000652\ H^3 + 27.5\ CG - 1{,}559$	275
$(Height)^3$, chest girth, and index of fat	$Y = 0.000616\ H^3 + 33.4\ CG - 184\ IF - 1{,}652$	270
	Expiratory reserve volume	
Height (140–160 cm.)	$Y = 8.9\ H - 786$	112
Height (160–190 cm.)	$Y = 15.9\ H - 1{,}914$	134
Height and actual weight	$Y = 12.5\ H + 0.5\ AW - 1{,}304$	125
$(Height)^3$	$Y = 0.000155\ H^3 + 25$	123
$(Height)^3$ and chest girth	$Y = 0.000102\ H^3 + 8.13\ CG - 357$	119
$(Height)^3$, chest girth, and index of fat	$Y = 0.000075\ H^3 + 12.7\ CG - 143\ IF - 429$	111
	Residual volume	
Height (140–160 cm.)	$Y = 11.1\ H - 1{,}055$	186
Height (160–190 cm.)	$Y = 16.7\ H - 1{,}920$	224
Height and actual weight	$Y = 12.5\ H + 2.91\ AW - 1{,}385$	199
$(Height)^3$	$Y = 0.000192\ H^3 - 45$	199
$(Height)^3$ and chest girth	$Y = 0.000123\ H^3 + 10.9\ CG - 567$	194
$(Height)^3$, chest girth, and index of fat	$Y = 0.000101\ H^3 + 14.3\ CG - 109\ IF - 612$	191

* The constants of the regression equations are based upon the following units of measurement: The lung volume and its subdivisions, ml.; height and chest girth, cm.; actual weight, Kg.

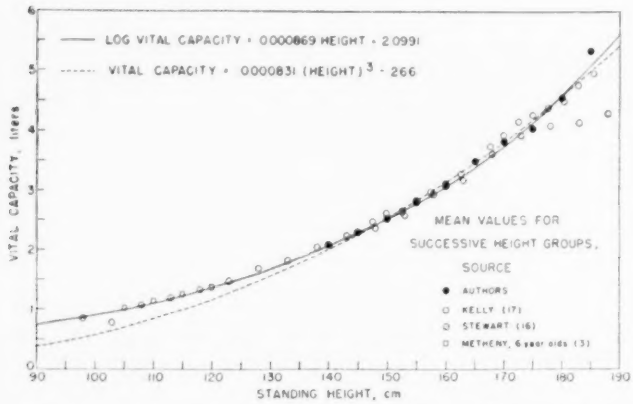

LOG VITAL CAPACITY = 0.000869 HEIGHT - 2.0991
VITAL CAPACITY = 0.000831 (HEIGHT)^3 - 266
VITAL CAPACITY, liters
STANDING HEIGHT, cm
MEAN VALUES FOR SUCCESSIVE HEIGHT GROUPS, SOURCE
AUTHORS
KELLY (17)
STEWART (16)
METHENY, 6 year olds (3)

FIG. 1. A COMPARISON OF THE SEMI-LOGARITHMIC CURVE WITH ONE BASED ON (HEIGHT)^3 AS A MEANS OF EXPRESSING THE RELATION OF VITAL CAPACITY TO STANDING HEIGHT THROUGHOUT CHILDHOOD AND ADOLESCENCE

The plotted points represent mean values for successive height groups taken from studies by Stewart (23), Kelly (24), Metheny (3), and the authors.

bx, was also investigated. This equation is the integral form of the differential equation, $\frac{dy}{dx} = by$. It has theoretical foundation since it is a fundamental expression of growth. If y and x represent lung volume and height, respectively, this equation would mean that an increase in lung volume due to an increase in height is a constant function of the lung volume existing at the moment. The curves of Figure 1 illustrate the two curvilinear relationships, semi-logarithmic and cubic, between vital capacity and height. Vital capacity was chosen as an example because more data from previous studies by other investigators are available for comparison. The curves were continued downward to the left, below the height range of the boys in the present study, to include standing heights as low as might be expected of young children old enough to cooperate in the test, for the purpose of testing, by comparison with the results of other investigators, whether either of the two curves were applicable to younger children. Two extensive studies of children by Stewart (23) and by Kelly (24) and a study of children three to six years of age by Metheny (3) were selected from the literature for comparison with the authors' data. In Figure 1 the mean values of vital capacity for the various height groups have been superimposed upon the regression curves which were fitted to the authors' data for boys of 10 to 17 years by the method of least squares.

Within the height range upon which the regression equations were based, 140–190 cm., the mean values of vital capacity found by Stewart and by Kelly for successive height groups agree well with either regression curve. Below this height range the curves separate. With few exceptions the mean values found by Stewart for younger children and those found by Metheny for six-year-olds agree well with the semi-logarithmic curve. The equation based on (height)^3 appears to give values that are too low in the height range below 140 cm. From a study of pre-school children, Metheny found that for a given height vital capacity increased with age. Mean values for children younger than six years would therefore lie below the semi-logarithmic curve. A recent study by Turner and McLean (4) gives values for the vital capacity of young children that lie for the most part above either curve.

The semi-logarithmic relations of the other subdivisions of the lung volume to standing height were also investigated. It was found that the correlation coefficients between height and the logarithms of the subdivisions of the lung volume tend to be slightly lower than those between height

and the non-logarithmic values and therefore add nothing to accuracy of prediction within the height range studied.

In the case of adults the relative proportions of the total lung capacity occupied by the different subdivisions have been shown to be more constant than the absolute volumes of the subdivisions (9, 22, 25–29). For the children of this study mean values for successive age groups, as well as the overall mean values for each subdivision, are given in Table V as percentages of the total lung capacity. For the group as a whole the various subdivisions show the following coefficients of variation: inspiratory capacity, 7.7 per cent; functional residual capacity, 13.6 per cent; residual volume, 22.9 per cent; expiratory reserve volume, 16.2 per cent; and vital capacity, 5.4 per cent. These values are only slightly lower than the corresponding coefficients of variation for the absolute volumes, which are obtained by referring the lowest standard error of estimate for each subdivision to the appropriate mean value for the whole group, 9.8, 16.0, 24.4, 16.5 and 8.5 per cent, respectively.

The data show no consistent change in the relative volumes of the various subdivisions which may be attributed to age. However, at 13 years and again at 15 years the functional residual capacity appears to occupy a smaller fraction of the total lung volume, due both to lower residual and expiratory reserve fractions. Some of the age differences are high enough to be statistically significant, for "t" values of 2.38, 2.85 and 2.33 were found for the decrease in functional residual capacity between 12 and 13 years, its increase between 13 and 14 years and its subsequent fall between 14 and 15 years, respectively. The decrease in the relative volume of the expiratory reserve between 14 and 15 years was also significant, with a "t" value of 3.44. Other differences are less significant and are more likely the result of chance sampling.[7]

Because of the apparent differences in the relative proportions of the subdivisions of the lung volume at 13 and 15 years, the effect of age upon the absolute volumes was investigated further. Figure 2 shows the distribution by age of the deviations of the determined values from those predicted from the (height)^3, chest girth and index of fat of each boy. The chart shows that certain boys of 13, 14 and 15 years had lower total lung capacities than were found in both younger and older boys and that in the case of the 14-year-old boys the lower lung capacity was related to a lower inspiratory capacity, while in the 13- and 14-year-old boys it was due largely to lower expiratory reserve and residual volumes. The data of Robin-

[7] The effect of the large experimental error which occurs in the determination of these volumes was considered in this connection. In the case of the residual volumes data from duplicate determinations are available and an estimation of the effect of the experimental error can therefore be gained by statistical analysis of variance (30). The greatest difference in the mean relative residual volume, 2.9 per cent units, was found between the 13- and 14-year-old groups. Although this difference did not prove to be significant because of the large individual variation, an analysis of variance was made in order to evaluate the relative effect of experimental and sampling errors. The 13- and 14-year-old groups included the results of duplicate determinations on 14 and 15 individuals respectively. The variance between groups was found to be 104.5, and that within groups was 44.6, while the variance between the results for duplicate determinations was 5.3. Thus, only a comparatively small portion of the large variance within groups can be attributed to the error of the technique.

TABLE V

Mean values of the subdivisions of the lung volume, expressed as percentages of the total lung capacity, for successive age groups from 11 to 17 years

Age	No. of cases	Inspiratory capacity	Functional residual capacity	Residual volume	Expiratory reserve volume	Vital capacity
yrs.		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
11	8	63.9±0.8*	36.1±0.8	17.9±1.4	18.2±1.1	82.1±1.4
12	8	61.6±1.9	38.5±1.9	20.4±2.0	18.1±1.3	79.6±2.0
13	16	66.7±1.2	33.3±1.2	17.0±1.0	16.3±0.7	83.0±1.0
14	20	62.0±1.1	38.0±1.1	19.9±1.0	18.1±0.4	80.1±0.8
15	23	65.4±0.9	34.6±0.9	18.9±0.8	15.7±0.4	81.1±0.8
16	13	62.7±1.6	37.3±1.6	20.5±1.4	16.8±0.4	79.5±1.4
17	5	62.8±1.3	37.2±1.3	19.3±1.3	17.8±0.6	80.7±1.3
11–17	93	63.9±0.5	36.1±0.5	19.1±0.5	17.0±0.3	80.9±0.5

* The figures following the ± signs represent the standard error of the mean.

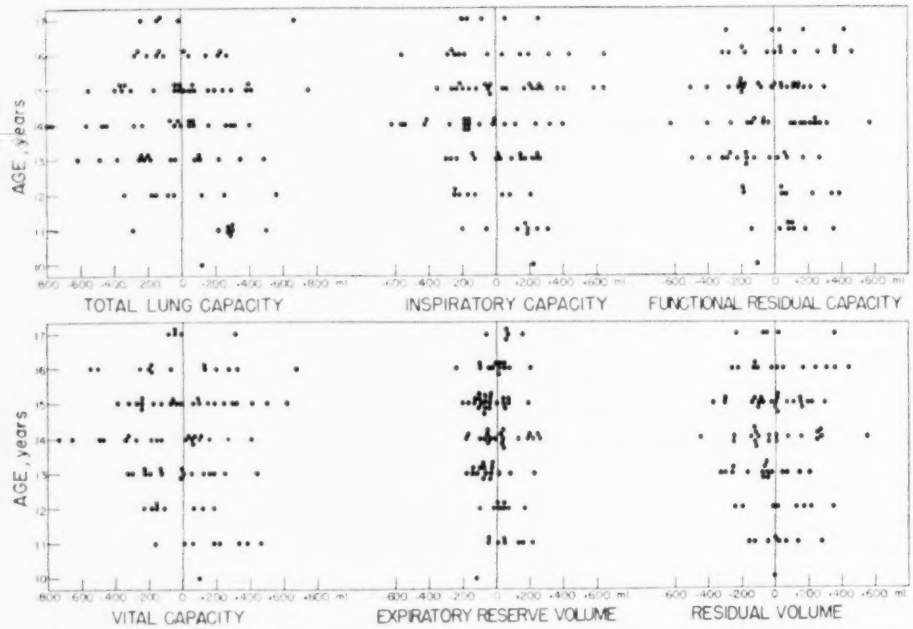


FIG. 2. DISTRIBUTIONS OF THE DEVIATIONS OF THE LUNG VOLUMES FOUND FROM THOSE PREDICTED FROM THE (HEIGHT)^3, CHEST GIRTH, AND INDEX OF FAT OF EACH INDIVIDUAL, PLOTTED ACCORDING TO AGE

son (5) also give evidence of an age effect upon the lung volume during puberty, since lower values were found for the total lung volume of the 15-year-old group as compared with those of the 10.5- and 17-year-old groups when the volumes were referred to a unit of body weight.

Kelly (24) found an effect of age or maturity in addition to those effects represented by (height)^3 and body weight when she determined partial correlation coefficients for vital capacity data from almost 4,000 boys between 12 and 18 years of age. Since the calculation of partial correlation coefficients from our data for boys 10 to 17 years of age showed no significant effect of age upon the vital capacity, apart from its effect upon body height and chest girth, it seemed possible that with this particular age range a decreasing effect associated with puberty may have masked a later maturing effect. Partial correlation coefficients were recalculated, omitting data for boys under 12 years in order to approximate the age range studied by Kelly. For the age range between 12 and 17 years the partial correlation between vital capacity and age, with (height)^3 held constant, was found to be 0.1830, a value high enough to be almost significant at the 5 per cent level ($t = 1.93$), but with chest girth held constant in addition to (height)^3 the correlation coefficient between vital capacity and age was reduced to 0.0038. Similar correlation coefficients between vital capacity, with (height)^3 and actual or normal weight held constant, were 0.1067 and 0.0084, respectively, and were therefore not significant.

DISCUSSION

Although body surface has sometimes been used as a standard of reference for purposes of estimating the normal vital capacity of an individual, standing height is now more commonly preferred. The use of (height)^3 was first suggested by Kelly (24) for the prediction of vital capacity and recently was advocated by Bateman (29) as a basis for the calculation of normal values for the total lung capacity and its other subdivisions. The present study gives further proof of its usefulness.

It is generally recognized that less variability is encountered when the volumes of the various subdivisions are expressed as percentages of the total lung capacity (9, 22, 25–29). By means of multiple regression equations containing (height)^3 and one or more other physical characteristics as variables it is possible to estimate absolute values which approximate relative values in accuracy. While statistical analysis of our data has given evidence of the predictive value of such multiple regression equations, it must be admitted that the individual values found for normal boys still deviate considerably from the calculated value, even when the best combination of physical characteristics is used. McCloy recognized this in his study of the vital capacity (18). The present results show that it is still more true of the other subdivisions of the lung volume.

Although individual anatomic, functional and psychological factors undoubtedly contribute to this variability, it is true also that errors in the methods used are comparatively large, even when all precautions are taken to insure accuracy. Vital capacity determinations are reasonably accurate, although proof of maximum effort is not entirely certain in many cases, especially in younger children. Separation of the vital capacity into two components, the inspiratory capacity and the expiratory reserve volume, depends upon determining the normal expiratory level. In the case of irregular breathing considerable error may be introduced in the measurement.[4] Errors in the determination of the functional residual or the residual volume by the closed circuit are even greater. Since this study was begun a number of improvements have been introduced to eliminate some sources of error (11–13, 31, 32). The open-circuit method introduced by Darling, Cournand and Richards (15), however, appears to allow fewer sources of error. It is hoped that this method may be applied to a further study of the lung volume in children.

Except for the vital capacity, which has been studied extensively, little information is available in the literature by which we may evaluate our results. The data of Robinson (5) are based upon an earlier modification of the closed-circuit method and are not therefore strictly comparable as regards the total lung capacity and those subdivisions which include the residual volume. Compared with values predicted from the mean height and weight of those age groups which fall within the age range of our study, Robinson found slightly higher values for all of the subdivisions of the lung volume, with deviations above the predicted values at 14 years of 64 , 102, and 116 ml. for inspiratory capacity, expiratory reserve and residual volumes, respectively. Lung volume studies of normal adults using the open-circuit method (28) or various modifications of the closed-circuit method (9, 22, 25–27, 32–34) cannot be compared with our data for boys because of the effect of age upon the various subdivisions and because in most cases the tests were conducted in the sitting posture (22, 27–29).

The question whether or not age exerts an effect upon the lung volume of the boy, apart from its effect upon body height and chest girth, during the years from 10 to 17 years cannot be answered conclusively from the results of this study. In the case of the vital capacity, Kelly's finding (24) of a significant correlation with age when (height)^3 and weight were held constant is contrary to the results of the present study which showed no significant correlation between vital capacity and age when (height)^3 and chest girth or weight were held constant. With data from comparatively few cases, as in the present study, the error due to chance sampling is greater. The indication that boys of 13, 14, and 15 years of age tend to have smaller lung volumes for a given (height)^3, chest girth and index of fat also raises the question of non-linearity of trend with age and the possibility of errors in the statistical analysis due to that source.

SUMMARY

The inspiratory capacity and the expiratory reserve volume in the resting recumbent state were determined by spirometric methods in 124 normal healthy boys of 10 to 17 years. In addition the residual lung volume was determined in 94 of these boys by the Lassen modification of the closed-circuit, oxygen dilution method of Christie. From these measurements the total lung capacity, the vital capacity and the functional residual capacity were calculated.

The results were analyzed by statistical methods in order to determine the effects of various physical characteristics of the individual upon these

volumes and to express these effects in the form of regression equations which may be used to estimate the "normal" lung volume and its subdivisions for a given individual. It was found that height or $(\text{height})^3$ is the best single standard of reference, but increased accuracy of prediction is gained by multiple regression equations including one or two standards of reference in addition to $(\text{height})^3$, with preference given to the index of build or chest girth and the index of fat rather than to actual or normal weight or to age.

For more limited ranges of height, linear equations which relate the lung volume and its subdivisions to standing height offer a convenient means of estimating normal values without much sacrifice of accuracy. Such equations are presented for two ranges of height, 140–160 cm. and 160–190 cm.

Semi-logarithmic equations which relate the logarithms of the lung volume and its subdivisions to standing height were also derived by statistical analysis of the data. While they offer no greater accuracy of prediction than the corresponding equations based upon $(\text{height})^3$ for the age and height range covered by the study, comparison with vital capacity data reported in the literature for younger children suggests that the semi-logarithmic equation may apply better to the larger range of ages covering early childhood to young adulthood.

In the resting recumbent position the relative proportions of the total lung capacity occupied by the inspiratory capacity and the expiratory reserve and residual volumes were found to be 63.9, 17.0 and 19.1 per cent, respectively, in boys of 10 to 17 years as a group. An effect of age upon the relative proportions of the total volume occupied by the various subdivisions during those years is suggested by relatively lower values for the expiratory reserve and residual volumes at 13 and 15 years. Certain of the 13-, 14- and 15-year-old boys were found to have lower total lung capacities relative to their size than were found in younger and older boys.

ACKNOWLEDGMENTS

The authors wish to express their deep appreciation and thanks to Dr. D. B. Dill, formerly of the Fatigue Laboratory at Harvard University, and at present Scientific Director, Medical Division, Army Chemical Center, Md., at whose suggestion the study was undertaken, and to Dr. Sid Robinson of the Department of Physiology, University of Indiana, who so kindly gave us the benefit of his experience with the methods.

REFERENCES

1. Morse, M., Schlutz, F. W., and Cassels, D. E., Relation of age to physiological responses of the older boy (10–17 years) to exercise. J. Appl. Physiol., 1949, **1**, 683.
2. Metheny, E., Breathing capacity during the first decade of life. A review of the literature on techniques, standards and clinical implications. J. Pediat., 1941, **19**, 841.
3. Metheny, E., Breathing Capacity and Grip Strength of Preschool Children. University of Iowa Studies in Child Welfare, Vol. XVIII, No. 2. Univ. of Iowa Press, Iowa City, 1940.
4. Turner, J. A., and McLean, R. L., Spirometric measurements of lung function in healthy children. Pediatrics, 1951, **7**, 360.
5. Robinson, S., Experimental studies of physical fitness in relation to age. Arbeitsphysiol., 1938, **10**, 251.
6. Lassen, H. C. A., Cournand, A., and Richards, D. W., Jr., Distribution of respiratory gases in a closed breathing circuit. I. In normal subjects. J. Clin. Invest., 1937, **16**, 1.
7. Christie, R. V., The lung volume and its subdivisions. I. Methods of measurement. J. Clin. Invest., 1932, **11**, 1099.
8. Standardization of definitions and symbols in respiratory physiology. Federation Proc., 1950, **9**, 602.
9. Hurtado, A., and Boller, C., Studies of the total pulmonary capacity and its subdivisions. I. Normal, absolute and relative values. J. Clin. Invest., 1933, **12**, 793.
10. Mainland, D., The Treatment of Clinical and Laboratory Data; An Introduction to Statistical Ideas and Methods for Medical and Dental Workers. Oliver & Boyd, Ltd., Edinburgh, 1938, p. 204.
11. Herrald, F. J. C., and McMichael, J., Determination of lung volume: simple constant volume modification of Christie's method. Proc. Roy. Soc., London, 1939, **126B**, 491.
12. McMichael, J., A rapid method of determining lung capacity. Clin. Sc., 1939, **4**, 167.
13. Meneely, G. R., and Kaltreider, N. L., Use of helium for determination of pulmonary capacity. Proc. Soc. Exper. Biol. & Med., 1941, **46**, 266.
14. Gilson, J. C., and Hugh-Jones, P., The measurement of the total lung volume and breathing capacity. Clin. Sc., 1949, **7**, 185.
15. Darling, R. C., Cournand, A., and Richards, D. W., Jr., Studies on the intra-pulmonary mixture of gases. III. An open circuit method for measuring residual air. J. Clin. Invest., 1940, **19**, 609.
16. Campbell, J. A., and Hill, L., Concerning the amount of nitrogen gas in the tissues and its removal by breathing almost pure oxygen. J. Physiol., 1931, **71**, 309.

17. McCloy, C. H., Appraising Physical Status: the Selection of Measurements. University of Iowa Studies in Child Welfare, Vol. XII, No. 2. Univ. of Iowa Press, Iowa City, 1936, p. 106.
18. McCloy, C. H., Appraising Physical Status: Methods and Norms. University of Iowa Studies in Child Welfare, Vol. XV, No. 2. Univ. of Iowa Press, Iowa City, 1938, pp. 14, 34, 52.
19. Boothby, W. M., and Sandiford, R. B., Nomographic charts for calculation of metabolic rate by the gasometer method. Boston M. & S. J., 1921, **185**, 337.
20. DuBois, D., and DuBois, E. G., Clinical calorimetry. X. A formula to estimate the approximate surface area if height and weight be known. Arch. Int. Med., 1916, **17**, 863.
21. Goulden, C. H., Methods of Statistical Analysis. John Wiley & Sons, Inc., New York, 1939, p. 211.
22. Aslett, E. A., Hart, P. D'A., and McMichael, J., The lung volume and its subdivisions in normal males. Proc. Roy. Soc., London, 1939, **126B**, 502.
23. Stewart, C. A., The vital capacity of the lungs of children in health and disease. Am. J. Dis. Child., 1922, **24**, 451.
24. Kelly, H. G., A Study of Individual Differences in Breathing Capacity in Relation to Some Physical Characteristics. University of Iowa Studies in Child Welfare, Vol. VII, No. 5. Univ. of Iowa Press, Iowa City, 1933.
25. Hurtado, A., Fray, W. W., Kaltreider, N. L., and Brooks, W. D. W., Studies of total pulmonary capacity and its subdivisions. V. Normal values in female subjects. J. Clin. Invest., 1934, **13**, 169.
26. Kaltreider, N. L., Fray, W. W., and Hyde, H. V., The effect of age on the total pulmonary capacity and its subdivisions. Am. Rev. Tuberc., 1938, **37**, 662.
27. Rotta, A., and Guerrero, F., Estudios sopre la capacidad pulmonar. An. Fac. Cienc. Méd. (Univ. Lima), 1936, **19**, 49.
28. Birath, G., Lung volume and ventilation efficiency. Changes in collapse-treated and non-collapse-treated pulmonary tuberculosis and in pulmonectomy and lobectomy. Acta. med. Scandinav., 1944, Supp. **154**, 1.
29. Bateman, J. B., Studies of lung volume and intrapulmonary mixing: normal lung capacities. J. Appl. Physiol., 1950, **3**, 133.
30. Goulden, C. H., *Op. cit.*, p. 122.
31. Izzo, R. A., and Chiodi, H., A modified Christie method for residual air measurements. Am. J. M. Sc., 1943, **206**, 190.
32. Meneely, G. R., and Kaltreider, N. L., The volume of the lung determined by helium dilution. Description of the method and comparison with other procedures. J. Clin. Invest., 1949, **28**, 129.
33. McMichael, J., and McGibbon, J. P., Postural changes in the lung volume. Clin. Sc., 1939, **4**, 175.
34. Whitfield, A. G. W., Waterhouse, J. A. H., and Arnott, W. M., The total lung volume and its subdivisions. A study in physiological norms. I. Basic data. Brit. J. Social Med., 1950, **4**, 1.

THE EFFECTS OF PITRESSIN ON THE METABOLISM AND EXCRETION OF WATER AND ELECTROLYTES IN NORMAL SUBJECTS AND PATIENTS WITH CIRRHOSIS AND ASCITES [1, 2]

By W. P. NELSON, III,[3, 4] AND L. G. WELT

(From the Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.)

(Submitted for publication November 23, 1951; accepted February 5, 1952)

INTRODUCTION

Salt and water are retained in nearly isotonic proportions in most edematous states. There is, however, a general tendency toward mild hypotonicity which is exaggerated when the salt intake is drastically reduced. This implies that there are forces operating to promote retention of water in excess of that which is secondary to the retention of salt. This primary retention of water may be due to *1*) an antidiuretic stimulus other than hypertonicity of the body fluids, *2*) an increased sensitivity to the normal amount of antidiuretic hormone, or *3*) an increased quantity of circulating antidiuretic hormone related to improper inactivation or excretion of this substance (1–3).

Previous studies (4–6) suggested that certain patients with edema were more sensitive than normal subjects to the prolonged administration of Pitressin. The present investigation was designed to determine *1*) whether patients with cirrhosis and ascites are more sensitive to endogenous or exogenous antidiuretic hormone, and *2*) whether these patients are unable to inactivate or eliminate these substances normally. The question of an increased secretion of posterior pituitary or other antidiuretic substance is considered by inference. The excretion of electrolytes was measured during the onset of a water diuresis, during sudden Pitressin antidiuresis, and during resumption of the water diuresis.

EXPERIMENTAL PROCEDURE AND METHODS

Eight normal subjects and seven patients with cirrhosis of the liver and ascites were studied. Food and water were restricted for 10–14 hours prior to the experiment which was started about 8 a.m. Each study was conducted with the subject in the recumbent position and nothing by mouth was permitted. All urines were voided specimens. Samples of blood were collected under oil at the start of each study.

Following a control period of 30 to 60 minutes, an infusion of 5 per cent glucose in water was started and continued throughout the experiment. Approximately 1,000 cc. were administered during the first hour. Thereafter, the rate of administration of the infusate was adjusted to attain and maintain a positive balance of 1 liter of water. The volume of infusate administered during a given period approximated the volume of urine voided plus the insensible loss of water which was assumed to be 40 cc./hr. This procedure promotes a relatively steady maximal rate of excretion of water due, presumably, to a continuous suppression of secretion of the antidiuretic hormone of the posterior pituitary gland. This may be regarded as a state of "physiological diabetes insipidus" (7).

After a steady maximal rate of excretion of water had been attained, Pitressin (Parke, Davis & Company, Lot No. 161), in doses of 2.5, 100, or 2,000 milliunits, was administered. The two smaller doses were administered intravenously, the largest dose subcutaneously. The rate of infusion was reduced proportionately to the antidiuretic response to the Pitressin, and the experiment was continued until either the initial maximal rate of excretion of water was attained, or until it appeared evident that a maximal diuresis, though lower than the initial rate, had been achieved.

The "time" in Tables IV, V, and VI refers to the number of minutes in the pre-Pitressin period of the study, and the number of minutes following administration of Pitressin in the second part of the study.

The chemical methods have been described in previous publications from this department (8, 9).

[1] Aided by an institutional grant and a research grant from the National Heart Institute, U. S. Public Health Service, and Contract V1001M–2300 of the Veterans Administration.

[2] Presented in abstract form at the 43rd Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., April 30, 1951.

[3] Work done during the tenure of a Life Insurance Medical Research Fellowship.

[4] Present address: Dept. of Internal Medicine, Veterans Administration Hospital, Albany, New York.

TABLE I

The effects on excretion of water of a water load and administration of Pitressin in normal subjects and patients with cirrhosis and ascites

		Time to maximal rate	Maximal rate	Dose of Pitressin	Minimum rate	Reduction in rate	Time for recovery
		min.	*cc./min.*	*milliunits*	*cc./min.*	*per cent*	*min.*
Normal subjects	P	90	15.8	2.5	7.9	50	88
	Del	122	7.9	2.5	2.7	66	88
	S	91	14.8	2.5	3.4	77	88
	H	72	14.1	2.5	4.7	67	87
	Co	83	15.6	100.	1.0	94	80
	V	62	30.0	100.	4.3	86	59
	Wa	88	24.4	2,000.	0.9	96	355+
	O	86	24.5	2,000.	3.9	85	273+
Patients with cirrhosis	R	65	2.7	2.5	1.2	86	117
	Des	60	23.8	2.5	10.4	56	54
	Cr	90	12.5	2.5	4.8	74	88
	Wo	89	2.7	100.	0.5	81	86
	B	75	21.7	100.	5.0	77	72
	R	97	2.2	2,000.	0.3	86	?
	Dup	95	7.5	2,000.	0.3	96	?

RESULTS

The average time required to reach a maximal rate of excretion of water was 87 minutes in the eight normal subjects, and 82 minutes in the seven studies of patients with cirrhosis and ascites (Table I). The normal subjects achieved the maximal rate between 62 and 91 minutes except for one who took 122 minutes. However, the earliest time of a maximal diuresis in this subject was probably masked by the irregular collections of urine. The patients with cirrhosis reached maximal rates between 60 and 95 minutes.

TABLE II

The concentration of Na, K, and Cl in the serum of the normal subjects and patients with cirrhosis and ascites prior to the experiment

Normal subjects				Patients with cirrhosis			
Subjects	Conc. in serum (*mEq./L.*)			Subjects	Conc. in serum (*mEq./L.*)		
	Na	K	Cl		Na	K	Cl
P	141.5	4.7	100.4	R	135.2	4.1	95.5
Del	141.0	—	99.1	Des	136.7	4.3	97.9
S	139.3	4.5	96.2	Cr	136.4	3.8	97.4
H	143.4	—	100.7	Wo	132.3	3.8	94.0
Co	137.5	5.0	98.0	B	137.8	4.4	104.8
V	140.6	4.0	100.4	R	—	—	—
Wa	141.2	3.8	99.6	Dup	131.8	4.4	—
O	139.1	4.2	96.8				

The average maximal rate of excretion of water was 18.4 cc./min. in the normal subjects as compared with an average of 10.7 cc./min. in the cirrhotics. However, the individual maximal rates varied from 7.9 to 30.0 cc./min. in the normals, and from 2.2 to 23.8 cc./min. in the cirrhotics. The three studies among the cirrhotics in which a maximum rate of excretion of water of less than 3 cc./min. was achieved were on patients who excreted a urine that was virtually free of salt. There was no *general* relationship, however, between either *1*) the maximal rate of excretion of salt and water, or *2*) the initial rate of excretion of salt and the maximal rate of excretion of water attained.

TABLE III

The relationship between initial concentration of sodium in serum, the maximum rate of excretion of water, and the initial and maximum rates of excretion of sodium attained by patients with cirrhosis and ascites

Subject	Conc. of Na in serum	Max. rate excretion of water	Initial rate excretion of Na	Max. rate excretion of Na
	mEq./L.	*cc./min.*	*microEq./min.*	*microEq./min.*
Dup	131.8	7.5	3.9	5.6
Wo	132.3	2.7	0.1	0.5
R	135.2	2.7	0.4	0.6
Cr	136.4	12.5	14.0	76.0
Des	136.7	23.8	—	148.0
B	137.8	21.7	—	191.0

TABLE IV

The effects on the rates of excretion of water and electrolytes of 2.5 milliunits of Pitressin in normal subjects and patients with cirrhosis and ascites

Normal subjects						Patients with cirrhosis					
Subject	Time	Rates of excretion				Subject	Time	Rates of excretion			
		H_2O	Na	Cl	K			H_2O	Na	Cl	K
	min.	*cc./min.*	*microEq./min.*				*min.*	*cc./min.*	*microEq./min.*		
P	−0	0.23	9	12	17	R	−0	0.07	0.	0.6	0.3
	+60	1.7	50	56	31		+65	0.90	0.5	2.6	13.0
	+90	12.3	167	186	92		+95	2.7	0.6	3.8	23.0
	+123	15.8	139	145	62		+125	2.5	0.5	5.5	21.0
	+150	14.4	94	98	42		+155	2.6	0.4	5.5	20.0
	Pitressin						Pitressin				
	+58	7.9	104	124	45		+55	1.3	0.3	4.0	15.0
	+88	10.5	73	84	44		+85	1.2	0.3	4.1	18.0
	+119	18.1	110	112	56		+117	1.3	0.3	4.3	20.0
							+146	2.6	0.4	5.7	23.0
							+175	2.4	0.4	5.8	22.0
Del	−0	0.5	—	79	36	Des	−0	—	—	—	—
	+92	1.0	74	75	24		+60	2.2	29.0	39.0	34.0
	+122	5.8	234	236	48		+85	23.4	122.0	131.0	44.0
	+152	7.9	256	255	38		+115	19.3	137.0	172.0	60.0
	+182	7.1	181	187	26		+148	23.8	148.0	188.0	67.0
	+212	6.9	160	165	26						
	Pitressin						Pitressin				
	+88	2.7	123	142	24		+26	15.7	121.0	174.0	64.0
	+118	6.0	164	170	23		+54	10.4	71.0	123.0	47.0
	+148	5.7	181	182	19		+86	23.6	73.0	113.0	40.0
							+116	16.7	47.0	73.0	37.0
							+141	13.0	39.0	87.0	36.0
S	−0	0.30	42	65	35	Cr	−0	0.46	14.0	27.0	19.0
	+60	1.4	109	135	53		+60	0.38	5.0	13.0	14.0
	+91	9.7	163	191	58		+90	8.8	66.0	113.0	65.0
	+120	14.7	160	166	49		+120	11.7	76.0	112.0	50.0
	+151	14.8	139	139	46		+150	12.5	71.0	115.0	53.0
							+180	11.8	71.0	119.0	53.0
	Pitressin						Pitressin				
	+57	3.4	86	112	35		+63	4.8	36.0	72.0	36.0
	+88	11.8	97	107	35		+88	7.8	14.0	47.0	31.0
	+117	13.3	97	93	31		+118	12.2	43.0	79.0	47.0
H	−0	0.6	64	79	52						
	+72	6.4	63	82	100						
	+102	14.1	78	72	127						
	+132	12.5	55	41	84						
	+162	12.8	64	40	67						
	+192	13.6	73	46	54						
	Pitressin										
	+57	4.7	55	41	22						
	+87	9.4	55	24	39						
	+117	11.0	50	19	24						
	+147	9.8	53	24	25						

However, in addition to the fact that the average concentration of sodium in the serum (Table II) was lower in the group of cirrhotics than in the normal subjects, there was an excellent correlation among the cirrhotics between this concentration and the maximal rate of excretion of water (Table III). The patients with the lowest concentrations of sodium in the serum excreted little salt and developed low maximal rates of excretion of water. These correlations were not evident in the group of normal subjects.

Two and a half milliunits of Pitressin reduced the average rate of flow of urine by 65 per cent in the four normal subjects and 72 per cent in the three patients with cirrhosis. The administration of 100 milliunits of Pitressin was followed by a 90 per cent reduction of urine flow in two normals as compared with 79 per cent in two cirrhotics. Two thousand milliunits of Pitressin administered subcutaneously reduced the rate of flow of urine by 90 and 91 per cent in two normal subjects and two patients with cirrhosis respectively (Table I).

The average time for recovery to a maximal rate of excretion of water following administration of 2.5 milliunits of Pitressin was 88 and 86 minutes in the normals and cirrhotics, respectively; following 100 milliunits it was 70 and 79 minutes. It will be noted that one each, of the normal subjects (Co) and of the patients with cirrhosis (B), failed to attain the pre-Pitressin maximal rate of excretion of water after the dose of 100 milliunits. However, they had both clearly recovered from the antidiuretic effect. Clear-cut *early* recovery was apparent in 232 minutes in the normal subjects following the subcutaneous administration of 2,000

TABLE V

The effects on the rates of excretion of water and electrolytes of 100 milliunits of Pitressin in normal subjects and patients with cirrhosis and ascites

Normal subjects						Patients with cirrhosis					
Subject	Time	Rates of excretion				Subject	Time	Rates of excretion			
		H_2O	Na	Cl	K			H_2O	Na	Cl	K
	min.	*cc./min.*	*microEq./min.*				*min.*	*cc./min.*	*microEq./min.*		
Co	−0	—	—	—	—	Wo	−0	0.28	0.1	0.8	20
	+83	4.9	44	137	102		+30	0.39	0.2	0.9	25
	+118	15.6	98	115	55		+59	1.1	0.5	1.9	48
	+148	15.6	95	107	48		+89	1.8	0.5	2.5	41
							+119	2.7	0.4	4.2	32
							+149	1.7	0.3	3.2	25
							+180	0.97	0.3	2.3	18
	Pitressin						Pitressin				
	+80	1.0	26	49	27		+56	0.54	0.2	2.5	16
	+116	7.7	62	121	83		+86	1.8	0.3	3.4	20
	+146	7.5	34	40	46		+116	2.7	0.3	4.6	20
	+176	6.8	34	29	31		+146	2.2	0.3	4.5	17
V	−0	0.81	190	207	64	B	−0	—	—	—	—
	+62	2.3	57	58	27		+60	1.2	31.0	19.0	33
	+86	30.0	432	390	162		+75	12.3	177.0	107.0	150
	+106	12.0	106	84	18		+90	21.7	191.0	100.0	104
	+127	25.2	219	159	33		+105	18.3	134.0	78.0	44
	Pitressin						Pitressin				
	+59	4.3	103	113	21		+17	10.0	116.0	91.0	51
	+80	21.2	265	268	61		+72	5.0	33.0	27.0	30
	+101	21.2	151	123	30		+106	8.4	27.0	23.0	37
							+143	7.3	26.0	16.0	30
							+183	9.2	34.0	14.0	33

TABLE VI

The effects on the rates of excretion of water and electrolytes of 2,000 milliunits of Pitressin in normal subjects and patients with cirrhosis and ascites

Normal subjects						Patients with cirrhosis					
Subject	Time	Rates of excretion				Subject	Time	Rates of excretion			
		H_2O	Na	Cl	K			H_2O	Na	Cl	K
	min.	*cc./min.*	*microEq./min.*				*min.*	*cc./min.*	*microEq./min.*		
Wa	−0	0.75	111	134	68	A	−0	0.33	0.4	1.5	24
	+54	1.7	120	142	84		+62	0.48	0.4	1.0	21
	+88	15.3	167	178	80		+97	1.2	0.5	2.6	23
	+115	24.4	305	266	73		+127	1.6	0.5	2.1	20
	+142	23.7	360	341	50		+157	1.8	0.5	3.6	19
							+189	2.2	0.6	3.7	20
							+217	1.5	0.5	4.5	20
	Pitressin						Pitressin				
	+37	11.8	264	250	38		+57	0.47	0.3	2.0	19
	+227	0.92	143	141	27		+87	0.43	0.4	1.9	22
	+300	3.6	120	128	22		+150	0.34	0.3	1.4	16
	+330	9.0	140	106	23		+210	0.37	0.3	1.7	13
	+355	11.4	164	98	22		+271	0.38	0.3	1.9	11
O	−0	0.38	42	81	66	Dup	−0	0.43	3.9	11.0	51
	+86	3.7	68	73	37		+65	0.78	4.6	10.0	48
	+110	18.2	144	159	69		+95	4.5	4.0	13.0	30
	+130	19.7	116	115	39		+125	7.5	5.6	20.0	33
	+142	23.5	153	121	40		+155	5.0	3.7	20.0	29
	+163	24.5	150	143	32						
	Pitressin						Pitressin				
	+62	6.5	137	146	56		+60	1.6	2.1	10.0	18
	+187	3.6	62	73	40		+150	0.33	1.7	7.0	16
	+232	10.2	70	73	48		+260	0.36	1.2	5.0	14
	+256	16.7	108	71	35		+300	0.38	1.2	4.0	14
	+273	20.9	123	72	31		+330	0.33	0.9	2.0	10

milliunits. In contrast, there was no evidence of recovery up to 271 and 330 minutes in the two cirrhotic patients who received this dose of Pitressin (Tables I, IV, V, and VI).

The rate of excretion of sodium and chloride increased sharply during the ascent of the initial diuresis in every experiment on a normal subject except for H (Tables IV, V, and VI). Although there was a tendency for the rates of excretion of these ions to increase in the patients with cirrhosis and ascites, this was unimpressive except in the studies on B and Cr. An increase in the rate of excretion of potassium was evident in almost every experiment, but the change was not striking. The antidiuretic period following the injection of Pitressin was characterized by a *decrease* in the rates of excretion of sodium and chloride in every subject, both normal and cirrhotic, except P. The rates of excretion of potassium decreased in most instances, but, again, these changes were small. In no instance in the post-Pitressin period did the rate of excretion of any of the three ions exceed the highest rate in the foreperiod.

The rates of excretion of these electrolytes remained the same or increased to a minor degree when the rate of excretion of water increased as the effect of Pitressin diminished. This is in sharp contrast to the augmented rates of excretion of salt during the development of the initial diuresis.

Glucosuria occurred commonly during the initial stages of the water diuresis in these studies. However, this was quantitatively insignificant and disappeared as the experiment progressed.

DISCUSSION

A decrease in the effective osmotic pressure of the body fluids promotes a graded suppression of the rate of secretion of antidiuretic hormone by the posterior pituitary gland; and this, of course, is associated with a reduction in the reabsorption of water in the distal tubules resulting in an augmented rate of excretion of water (*7*). This, however, is not limitless; beyond a certain degree of dilution of the body fluids a further increase in the positive balance of water will not promote a greater rate of excretion (10). The interval between the induction of adequate dilution of the body fluids and the attainment of the maximal rate of excretion of water represents the time necessary to inactivate or eliminate the circulating antidiuretic hormone. The fact that the average time required to attain a maximal rate of excretion of water did not differ in these two groups of subjects implies that patients with cirrhosis and ascites have no deficiency with respect to the inactivation or elimination of that antidiuretic hormone whose secretion is suppressed by dilution of the body fluids. The degree of antidiuresis induced by a particular dose of Pitressin was the same in each group. This can only mean that the cells of the distal tubules of the kidneys of patients with cirrhosis and ascites are not more sensitive to a given level of circulating Pitressin. The rates of recovery from the Pitressin antidiuresis were the same in the two groups when doses of Pitressin that may be considered within the physiological range were administered (11). Essentially similar results have been reported by White, Rubin, and Leiter (12). There was, however, a distinct delay in recovery in the two subjects with cirrhosis when the largest dose (2,000 milliunits) was administered. It may be concluded, therefore, that the cirrhotic is able to inactivate or eliminate exogenously administered antidiuretic hormone in doses within physiological limits.

There remain two distinct differences between the two groups of subjects: *1*) the lower maximal rate of excretion of water, and *2*) a delayed recovery from the antidiuresis induced by a large dose of Pitressin. It was mentioned in the preceding section that the concentration of sodium in the serum was lower in the patients with cirrhosis, and, in this group, there was a clear correlation between the lower concentration of this ion in the serum and a low maximal rate of excretion of water. It is most probable in this instance that the depressed concentration of sodium in the serum is a result and not the cause of an inability to excrete water at a normal rate. It may be highly relevant, however, that the patients with extremely depressed rates of excretion of salt were the same who exhibited low maximal rates of diuresis. It suggests the possibility that both the antisaluresis and antidiuresis derive from a *common* stimulus.

The evidence is clear (1–3) that a contracted plasma volume, or some function thereof, serves as a stimulus to promote an increased reabsorption of salt by the renal tubules. Moreover, there is a considerable body of data to support the contention that a contracted volume may promote a retention of water in excess of that which is related to the increased reabsorption of salt. The precise mechanism of the antidiuresis associated with a contracted plasma volume is not clear, but the evidence at this time is most compatible with the hypothesis that the contracted volume promotes the secretion of the antidiuretic hormone of the posterior pituitary gland (13–17).

A progressive increase in the volume of an ascitic collection implies that fluid is transuding from the blood stream more rapidly than it is reabsorbed. This presents the stimulus of a contracted plasma volume which may be responsible for both the antisaluresis and antidiuresis seen among some of these patients with cirrhosis and ascites. When the body fluids of the patient with cirrhosis are diluted by the production of a positive water balance, secretion of the hormone of the posterior pituitary gland will not be completely suppressed since this procedure does not modify the contracted plasma volume which remains as a persistent stimulus for secretion of antidiuretic hormone. Thus, although this patient will have no delay in achieving a maximal diuresis, the magnitude of this diuresis will be less than normal in proportion to the intensity of the stimulus provided by the contracted plasma volume.

A delay in recovery from the antidiuresis provoked by the administration of Pitressin must be interpreted to mean that an effective antidiuretic concentration of this hormone has been maintained for a longer time. It is clear that there is such a delay in recovery in the normal subjects who re-

ceived the dose of 2,000 milliunits compared with other normals who received much smaller doses. This may be related both to the size of the dose and the different route of administration. The large dose and slower access to the circulation may provide an adequate antidiuretic concentration for a longer period of time. The rate of reabsorption from a subcutaneous depot may well be slower in a patient with hypoalbuminemia and this may account for the greater delay in recovery by the cirrhotics.

Several alternative suggestions to explain this difference may be offered. When Pitressin is administered to a normal subject with suppression of secretion of antidiuretic hormone induced by hypotonicity of the body fluids, the mechanisms involved in the inactivation or excretion of this hormone are completely unsaturated. This is not the case in the cirrhotic with ascites if the contracted plasma volume has not been corrected. It may be that partial saturation of the mechanisms involved in elimination of antidiuretic hormone may make it more difficult to eliminate completely such a large dose.

It has been alternatively suggested that the diseased liver is deficient with respect to inactivation of antidiuretic hormone, and that the increased quantities of antidiuretic substance and consequent ascites are related to this dysfunction (18–20). However, the degree of liver disease and ascites formation are not necessarily well correlated. Moreover, the retention of salt and water may be mitigated if a communication is made between the ascitic cavity and subcutaneous tissue of a patient with cirrhosis and ascites (21–23). There is no evidence that this procedure modifies the disease in the liver. In addition, there appears to be a similar abnormal response to administered posterior pituitary substance in patients with congestive heart failure and the nephrotic syndrome (5). There would, therefore, appear to be very little reason to indict the diseased liver *per se* for this delay in recovery from the antidiuresis.

The administration of Pitressin did not promote an increase in the rate of excretion of sodium, chloride, or potassium in either group. In most instances there were no changes or minimal decreases in these rates of excretion. A similar lack of effect of Pitressin on the rates of excretion of electrolytes has been reported by others (12, 24, 25). The sharp increase in the rates of excretion of sodium and chloride with the ascent of the initial diuresis, and the almost complete absence of this response during the development of the post-Pitressin diuresis deserve consideration. There are numerous reports of an increase in the rate of excretion of salt associated with the onset of a water diuresis. This has most frequently been interpreted to represent an effect of the increasing urine flow *per se*. Crutchfield and Wood (26) made the interesting observation that the acceleration in the rate of excretion of salt associated with a water diuresis was inversely correlated with the rate of urine flow prior to the administration of the load of water. It is well recognized that there is an increased tubular reabsorption of sodium chloride in water deprivation. The rate of excretion of sodium may be quite low in patients suffering with severe deficits of water despite the fact that the concentration of sodium in the serum is distinctly elevated. This has been called the "dehydration reaction" (9, 27). It would, therefore, be anticipated that the state of hydropenia should be characterized by some increase in the renal tubular reabsorption of salt. The administration of a load of water corrects the hydropenic state which would be expected to suppress the stimulus favoring the reabsorption of salt and promote an increase in its rate of excretion. Later in the course of the diuresis the excretion of salt diminishes which may be related to a decrease in the concentration of sodium and chloride in the serum. From the time of the administration of Pitressin to the end of the study the positive balance of water was maintained at a reasonably constant level. Therefore, under these circumstances the post-Pitressin diuresis developed at a time when hydration was more than adequate and was unchanging. This second diuresis was not associated with a relief from dehydration; there was no alteration that would modify salt reabsorption, and therefore, there was little or no increase in its rate of excretion.

SUMMARY AND CONCLUSIONS

1. Normal subjects and patients with cirrhosis and ascites did not differ *a*) in the time required to achieve a maximal rate of diuresis in response to the administration of a water load, or *b*) in the intensity or duration of the antidiuresis promoted

by a dose of 2.5 or 100 milliunits of Pitressin. It is therefore concluded that the patient with cirrhosis and ascites displays no evidence of an increased sensitivity to or an inability to eliminate or inactivate endogenous or physiologic doses of exogenous posterior pituitary hormone.

2. The rate of maximal diuresis was quite low in some of the patients with cirrhosis and ascites. This was associated with a markedly depressed rate of excretion of salt. Since a contracted plasma volume may promote the secretion of antidiuretic hormone and salt retention, it is suggested that this single stimulus may be responsible for the depression of both rates of excretion.

3. The cause of the delayed recovery from the antidiuresis following the largest dose of Pitressin in the cirrhotic may be explained by a slower rate of absorption of the hormone from the subcutaneous tissues into the circulation. This might allow a longer interval during which an adequate antidiuretic concentration obtained. Alternative suggestions are offered.

4. The administration of Pitressin did not promote the excretion of sodium chloride.

5. The rates of excretion of sodium and chloride increased significantly during the initial period of the diuresis in the normal subjects. However, there were little or no changes in these rates during the diureses following recovery from the Pitressin. The administration of the water load corrected the deficit of the hydropenic state initially, thus suppressing a stimulus favoring a low rate of excretion of salt. Since there were no alterations in the volume or tonicity of the body fluids throughout the remainder of the study, there were no significant influences that would tend to modify the rate of excretion of salt.

ACKNOWLEDGMENT

The authors are indebted to Jane V. Lee, A.B., and Gloria E. Nassif, A.B., for their aid in these studies.

REFERENCES

1. Peters, J. P., The role of sodium in the production of edema. New England J. Med., 1948, **239**, 353.
2. Welt, L. G., and Seldin, D. W., The pathologic physiology and treatment of edema. V. A. Tech. Bull., Jan. 12, 1951, TB 10-69.
3. Peters, J. P., Sodium, water, and edema. J. Mt. Sinai Hosp., 1950, **17**, 159.
4. Lloyd, C. W., Harris, J. F., and Lobetsky, J., Some studies of posterior pituitary and adrenal cortical interrelationships. J. Clin. Invest., 1951, **30**, 657.
5. Welt, L. G., Seldin, D. W., and Cort, J. H., The effects of the pituitary and adrenal hormones on the metabolism and excretion of sodium and water. J. Clin. Invest., 1951, **30**, 682.
6. Welt, L. G., and Seldin, D. W., The effects of the chronic administration of Pitressin in normals and patients with cirrhosis and ascites. In preparation.
7. Verney, E. B., Croonian lecture; The antidiuretic hormone and the factors which determine its release. Proc. Roy. Soc. London, 1947, s.B., **135**, 25.
8. Hald, P. M., The flame photometer for the measurement of sodium and potassium in biological materials. J. Biol. Chem., 1947, **167**, 499.
9. Elkinton, J. R., and Taffel, M., Prolonged water deprivation in the dog. J. Clin. Invest., 1942, **21**, 787.
10. Welt, L. G., and Nelson, W. P., III, Observations on the excretion of water. J. Applied Physiol., in press.
11. Lauson, H. D., The problem of estimating the rate of secretion of antidiuretic hormone in man. Am. J. Med., 1951, **11**, 135.
12. White, A. G., Rubin, G., and Leiter, L., Studies in edema. III. The effect of Pitressin on the renal excretion of water and electrolytes in patients with and without liver disease. J. Clin. Invest., 1951, **30**, 1287.
13. Martin, S. J., Herrlich, H. C., and Fazekas, J. F., Relation between electrolyte imbalance and excretion of an antidiuretic substance in adrenalectomized cats. Am. J. Physiol., 1939, **127**, 51.
14. Leaf, A., and Mamby, A. R., An antidiuretic mechanism not regulated by extracellular fluid tonicity. J. Clin. Invest., 1951, **30**, 654; 1952, **31**, 60.
15. Brun, C., Knudsen, E. O. E., and Raaschou, F., The influence of posture on the kidney function. I. The fall of the diuresis in the erect posture; II. Glomerular dynamics in the passive erect posture. Acta med. Scandinav., 1945, **122**, 315; 332.
16. Welt, L. G., and Orloff, J., The effects of an increase in plasma volume on the metabolism and excretion of water and electrolytes by normal subjects. J. Clin. Invest., 1951, **30**, 751.
17. Judson, W. E., Epstein, F. H., Tinsley, C. M., Burrows, B. A., and Wilkins, R. W., The hemodynamic and renal functional effects of venous congestion of the limbs in patients with diabetes insipidus. J. Clin. Invest., 1950, **29**, 826.
18. Ralli, E. P., Robson, J. S., Clarke, D., and Hoagland, C. L., Factors influencing ascites in patients with cirrhosis of the liver. J. Clin. Invest., 1945, **24**, 316.
19. Heller, H., and Urban, F. F., The fate of antidiuretic principle of post-pituitary extracts *in vivo* and *in vitro*. J. Physiol., 1935, **85**, 502.
20. Eversole, W. J., Birnie, J. H., and Gaunt, R., Inactivation of posterior pituitary antidiuretic hormone by the liver. J. Clin. Endocrinol., 1948, **8**, 616.

21. Peters, J. P., The problem of cardiac edema. Am. J. Med., 1952, **12**, 66.
22. Crosby, R. C., and Cooney, E. A., Surgical treatment of ascites. New England J. Med., 1946, **235**, 581.
23. Parker, M. L., and Breckler, I. A., Contribution to the surgical treatment of ascites. Modification of the Crosby-Cooney technique. J. A. M. A., 1950, **144**, 1091.
24. Murphy, R. J. F., and Stead, E. A., Jr., Effects of exogenous and endogenous posterior pituitary antidiuretic hormone on water and electrolyte excretion. J. Clin. Invest., 1951, **30**, 1055.
25. Chalmers, T. M., Lewis, A. A. G., and Pawan, G. L. S., Effect of posterior pituitary extracts on the renal excretion of sodium and chloride in man. J. Physiol., 1951, **112**, 238.
26. Crutchfield, A. J., Jr., and Wood, J. E., Jr., Urine volume and total renal sodium excretion during water diuresis. Ann. Int. Med., 1948, **28**, 28.
27. Winkler, A. W., Danowski, T. S., Elkinton, J. R., and Peters, J. P., Electrolyte and fluid studies during water deprivation and starvation in human subjects, and effect of ingestion of fish, of carbohydrate, and of salt solutions. J. Clin. Invest., 1944, **23**, 807.

DIURNAL VARIATION IN THE DIURETIC RESPONSE TO INGESTED WATER

By SOLOMON PAPPER[1] AND JACK D. ROSENBAUM

(From the Thorndike Memorial Laboratory, Second and Fourth [Harvard] Medical Services, Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston; and the Medical Service and Research Laboratory, Cushing Veterans Administration Hospital, Framingham, Mass.)

(Submitted for publication December 15, 1951; accepted February 5, 1952)

In normal individuals the renal excretion of water, sodium, potassium, and uric acid is less at night than during the day. This diurnal variation persists when no food or fluids are taken as well as when identical quantities of food, fluid, and electrolyte are ingested at regular intervals throughout the 24-hour period and is not easily altered by changes in the cycle of sleep and activity (1–12). The nocturnal decrease in excretion of water, sodium, and chloride occurs in spite of recumbent posture, the assumption of which in the daytime tends to augment the urinary output of these substances (6, 10, 13–17).

The mechanisms involved in the maintenance of the normal diurnal excretory rhythm are obscure. Kleitman suggested decreased nocturnal arterial pressure as a factor (4). Simpson postulated that the low excretion of chloride at night might be related to the acidosis of sleep (18). Stanbury and Thomson noted that the diurnal rhythm appeared to be intimately linked with a simultaneously occurring cycle of acid or alkali excretion (19). Observations in patients with Addison's disease and in individuals receiving cortisone therapy indicate that the adrenal cortical hormones may modify the diurnal excretory rhythm (20–22). Sirota, Baldwin, and Villarreal have demonstrated that the small nocturnal decrease in glomerular filtration rate is of insufficient magnitude to account for nocturnal oliguria, which must therefore depend upon increased tubular reabsorption of water (11).

Blomhert, observing that water loads administered to normal subjects at night evoked a smaller diuresis than did similar loads by day, postulated increased secretion of antidiuretic hormone at night (23). Under the conditions of his experiments, not only was less water excreted at night, but the rates of sodium and chloride excretion were also much lower than by day. Since it has been shown that the rate of urine flow under conditions of "maximal" water diuresis is related to, and perhaps dependent upon, the concomitant rate of electrolyte excretion (24), it is possible that the diurnal differences in water diuresis noted by Blomhert were secondary to the diurnal rhythm of solute excretion, however the latter may be determined. It seemed important therefore to study water diuresis under conditions such that sodium and chloride excretion were of similar magnitude at night and by day.

EXPERIMENTAL PROCEDURE AND METHODS

Three young adult male volunteers without evidence of renal or cardiovascular disease served as subjects. The response to administration of a water load was studied four times in each subject: once by day and once at night when a diet poor in sodium was being taken and once again both by day and at night when sodium intake was much larger. Since with a constant intake of sodium at any level, its nocturnal excretion tends to be less than that of the day, an effort was made to achieve excretion rates of comparable magnitude. This was accomplished by carrying out the first study at night, 48 hours after the subject had been taking a diet in which the daily sodium intake was limited to 35 meq., and the second study by day after he had remained on the same diet for an additional 48 hours. The intake of sodium chloride was then increased by giving 40 meq. orally as 0.9 per cent saline solution at 6 a.m., noon, 6 p.m. and midnight each day and the studies repeated after several days.

The nocturnal experiments were begun at about midnight without antecedent fluid restriction and were preceded by three to four hours of sleep in Subjects I and II. Subject III remained in bed after 9 p.m. but did not sleep. The daytime experiments were begun between 8 and 9 a.m. after approximately eight hours of sleep followed by a light breakfast at 7 a.m. During each experimental period the subject was awake in a well lighted

[1] United States Public Health Service Postdoctorate Research Fellow in Medicine, Thorndike Memorial Laboratory and Department of Medicine, Harvard Medical School, Boston, Mass.

room and remained recumbent throughout except to urinate. The minimum interval between successive studies was 36 hours. At the beginning of each experiment the subject voided, was weighed and then drank 20 ml. of tap water per kilogram of body weight within a period of eight to 15 minutes. The resultant water load was maintained for at least two hours after the onset of maximal diuresis by administering sufficient water after each voiding to maintain constant weight throughout the experimental period. Voided urine was collected at intervals of 30 minutes or less after diuresis had begun. Samples of venous blood were taken before water loading and during the period of maximal urine flow.

Sodium and potassium were determined by means of an internal standard flame photometer. Chloride was determined in the urine by the Volhard-Harvey titration (25) and in the serum by the Hald-Patterson technique (26) or the Van Slyke and Hiller modification of the Sendroy procedure (27). The method of Peters (28) was employed for determination of creatinine in urine; for serum the Hare method (29) was utilized.

RESULTS

The onset of diuresis was delayed at night as contrasted to the day in each of the three subjects, irrespective of salt intake (Table I, Figure 1).

On the second day of the salt-poor regimen, the excretion rates of sodium and chloride at night were slightly higher than those of the daytime studies carried out on the fourth day of salt restriction. Nevertheless the volume of urine excreted during the two-hour period of maximal diuresis at night was slightly less than that excreted during the day. The concentrations of sodium and chloride were necessarily higher in the nocturnal urine.

When the sodium chloride intake was 195 meq. daily, the urine volume, excretion rates of sodium and chloride and urinary concentrations of these ions were all much greater during the day than at night.

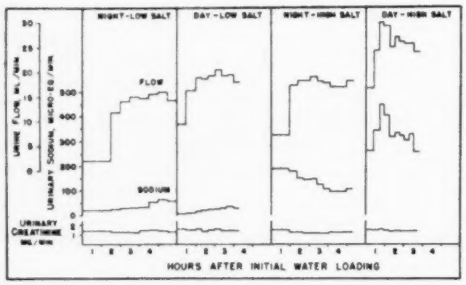


FIG. 1. RATES OF URINE FLOW, SODIUM EXCRETION, AND CREATININE EXCRETION DURING DAY AND NIGHT WATER LOADS UNDER CONDITIONS OF LOW SALT AND HIGH SALT DIETS IN SUBJECT II

TABLE I

Renal excretion of water and electrolytes and serum electrolyte concentrations during constant water load by day and at night

Subject and diet	Time	Onset of maximal diuresis (*minutes after loading*)	Urine*							Serum†			Creatinine‡	
			Volume	Sodium		Potassium		Chloride		Sodium	Potassium	Chloride	U/P	Clearance
			ml.	*meq.*	*meq./L.*	*meq.*	*meq./L.*	*meq.*	*meq./L.*	*meq./L.*	*meq./L.*	*meq./L.*		*ml./min.*
I	Night	65	1,130	6.4	5.6	9.1	8.0	8.1	7.1	137	4.3	103	15.5	144
Low salt	Day	53	1,330	5.2	3.9	21.3	16.0	6.5	4.8	137	4.2	101	12.1	137
II	Night	110	1,775	4.3	2.4	3.7	2.0	1.0	0.5	140	4.2	102	14.5	144
Low salt	Day	55	2,335	3.1	1.3	15.6	6.7	2.0	0.8	140	4.2	103	11.2	200
III	Night	115	1,230	5.0	4.0	7.2	5.8	3.1	2.5	—	—	—	—	—
Low salt	Day	47	1,397	3.3	2.3	10.8	7.7	5.0	3.5	138	4.0	100	13.8	143
I	Night	76	885	7.8	8.8	4.2	4.7	7.2	8.1	140	4.1	111	20.9	153
High salt	Day	58	1,684	28.4	16.8	19.5	11.6	30.5	18.1	138	4.2	106	14.7	181
II	Night	71	2,175	15.4	7.0	8.2	3.7	12.0	5.5	143	4.2	105	8.9	161
High salt	Day	37	3,193	41.0	12.9	18.2	5.7	40.9	12.8	139	4.1	105	5.5	162
III	Night	85	888	6.7	7.5	6.6	7.4	6.5	7.3	137	4.4	104	16.3	123
High salt	Day	57	1,711	15.6	9.1	14.7	8.6	20.0	11.6	139	4.3	105	12.9	160

* Values are for the two-hour period of maximal diuresis.
† Pre-loading values.
‡ 30 minute collection period during maximal diuresis.

Although in some instances endogenous creatinine clearances were higher by day and on the higher salt diet, this pattern was inconsistent.

The ratios of the concentrations of creatinine in the urine to those in the plasma (U/P) were consistently higher at night than by day, indicating that a larger fraction of filtered water was reabsorbed by the tubules at night, irrespective of salt intake.

Excretion of potassium was always much greater during the day than at night. In order to determine whether the difference in potassium excretion might explain the nocturnal oliguria, Subject I, while taking the diet poor in sodium, ingested 67 meq. of potassium chloride over a period of one hour and 40 minutes at the height of a nocturnal diuresis. Prior to the administration of potassium chloride, a rate of urine flow of 9.3–9.6 ml./min. had been achieved (Figure 2). At this time, the rate of potassium excretion was 58–73 microEq./min. and that of sodium was 40–47 microEq./min. Following the ingestion of potassium, its excretion reached a height of 301 microEq./min., associated with an augmentation in the rates of sodium and water excretion to 129 microEq./min. and 11.0 ml./min., respectively. During subsequent collection periods, although potassium excretion remained high, the excretion rates of water and sodium declined to below their initial magnitudes. Thus, augmentation of nocturnal water diuresis took place only during the periods when sodium excretion was also greatly increased.

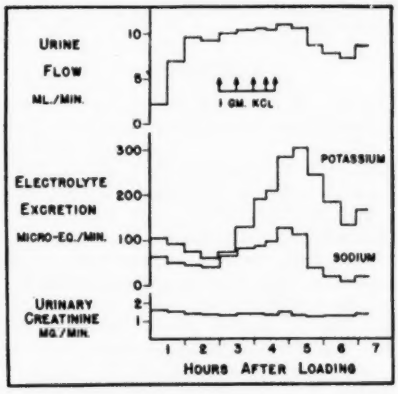


FIG. 2. NOCTURNAL POTASSIUM LOAD DURING "MAXIMAL" WATER DIURESIS (SUBJECT I)

While the subject was taking the low sodium diet, his maximal potassium excretion during the day was 191 microEq./min. and was associated with a sodium excretion of 47 microEq./min. and a urine flow of 11.5 ml./min. During the night study the rate of urine flow was 8.7 ml./min. at a time when the potassium excretion was 244 microEq./min. and the sodium, 36 microEq./min. Hence, despite a far greater rate of potassium excretion at night in this experiment, the rate of urine flow remained less than by day, except during the period of natriuresis.

While Subject II was on the low salt diet, urea excretion was determined night and day during the period of maximal diuresis. The night and day values were 0.414 mM./min. and 0.437 mM./min., respectively. Thus, the difference between the night and day response to water loading appears to be unrelated to differences in urea excretion.

DISCUSSION

Three general mechanisms which might be responsible for the decrease in urine flow which usually occurs at night even when the intake of food, fluid and electrolyte is equally distributed throughout the day and night, require consideration. These are: *1*) decreased glomerular filtration rate at night; *2*) decreased sodium excretion and, related to it, decreased urine flow at night; and *3*) increased tubular reabsorption of water due to higher levels of activity of antidiuretic hormone (ADH) at night. The available evidence would indicate that the diurnal differences in filtration rate cannot explain the nocturnal suppression of urine flow (11). Our observations that urine flow is less at night in subjects on a salt-poor regimen, under such conditions that nocturnal rates of sodium excretion were slightly higher than those of the day, indicate that the second mechanism cannot under these conditions be responsible. This suggests that increased tubular reabsorption of water alone, presumably as a result of increased ADH activity at night, might be important.[2]

[2] Such increased ADH activity could be due to increased secretion, increased sensitivity of the renal tubules to its action or a decrease in its dissipation. The first hypothesis is the most attractive since under physiologic conditions the rate of ADH secretion is known to vary greatly, whereas the other two phenomena have not been demonstrated.

Findings consistent with this hypothesis include: *1)* higher nocturnal creatinine U/P ratios; *2)* higher urinary sodium and chloride concentrations at night; and *3)* delay in onset of nocturnal diuresis. Delay in the onset of diuresis after water loading has been shown by Burn and Grewal to be quantitatively related to the size of a dose of exogenous posterior pituitary extract (30).

In the studies conducted while the subjects were taking 195 meq. of sodium daily, far less sodium and chloride were excreted at night than by day, with marked increase in the difference between night and day rates of urine flow. That this difference in flow is related chiefly to salt excretion rather than to increased ADH activity is suggested by the fact that sodium, chloride and potassium concentrations in the urine were all lower at night. However, the fact that the time interval between water loading and attainment of maximal flow was greater by night than by day in these experiments also indicates that the level of ADH activity prior to loading was higher at night (30).

The observations described provide no information concerning the basic mechanisms which determine the diurnal rhythm of solute excretion. They do indicate, however, that the diurnal rhythm of water excretion is related both to the excretory rhythm for solute and to diurnal variations in activity of antidiuretic hormone. The former appears to be of greater quantitative significance when salt intake is plentiful. Moreover the relation of the rhythm of water excretion to that of sodium seems clear whereas no association was apparent with the excretion of potassium or urea.

Of interest is the observation that, when the subjects were taking the diet containing 195 meq. of sodium, a sharp increase in sodium excretion took place after the morning water load (Figure 1). This was not observed in the nocturnal experiments. On the contrary, there was a progressive decline in sodium excretion. When the diet was poor in sodium, no such prompt natriuresis occurred either at night or by day. These observations may indicate that under conditions when salt is plentiful, expansion of the volume of body water in the morning is an effective stimulus for significantly increased sodium excretion. It has been demonstrated that the natriuretic response of the kidney to hypotonic expansion of extracellular fluid volume is modified by the posture of the subject (31). It is possible that this response to increased volume is also influenced by the time of day at which the expansion occurs.

CONCLUSIONS

1. Water loads of 20 ml. per kilogram of body weight were instituted and maintained by day and at night in three subjects while they were receiving a diet containing 35 meq. of sodium daily and again when the intake was 195 meq. daily.

2. While the subjects were on the low salt diet during periods in which similar night and day sodium excretion was achieved, definite but slight inhibition of nocturnal water diuresis was demonstrated.

3. When the subjects were on the diet of higher salt content, sodium excretion during the water diuresis was much less at night than during the day. Under these conditions the difference between night and day rates of urine flow was of very large magnitude.

4. It seems probable that the diminished diuretic response to water loading at night is related both to increased nocturnal secretion of antidiuretic hormone and to decreased renal excretion of sodium. The latter appears to be the more important factor when salt intake is liberal.

ACKNOWLEDGMENTS

The technical assistance of Miss Miriam Halpin, Miss Elsie C. Rossmeisl and Mrs. K. T. Yen is gratefully acknowledged.

The authors wish to express their appreciation to Dr. Maurice B. Strauss for his suggestions and advice in preparing this manuscript.

REFERENCES

1. Leathes, J. B., On diurnal and nocturnal variations in the excretion of uric acid. J. Physiol., 1906, **35**, 125.
2. Cathcart, E. P., Kennaway, E. L., and Leathes, J. B., On the origin of endogenous uric acid. Quart. J. Med., 1907–8, **1**, 416.
3. Campbell, J. A., and Webster, T. A., Day and night urine during complete rest, laboratory routine, light muscular work and oxygen administration. Biochem. J., 1921, **15**, 660.
4. Kleitman, N., Studies on the physiology of sleep. I. The effects of prolonged sleeplessness on man. Am. J. Physiol., 1923, **66**, 67.
5. Simpson, G. E., Diurnal variations in the rate of urine excretion for two hour intervals; some associated factors. J. Biol. Chem., 1924, **59**, 107.

6. Bazett, H. C., Thurlow, S., Crowell, C., and Stewart, W., Studies on the effects of baths on man. II. The diuresis caused by warm baths, together with some observations on urinary tides. Am. J. Physiol., 1924, **70**, 430.
7. Manchester, R. C., The diurnal rhythm in water and mineral exchange. J. Clin. Invest., 1933, **12**, 995.
8. Jores, A., Physiologie und Pathologie der 24-Stunden-Rhythmik des Menschen. Ergebn. d. inn. Med. u. Kinderh., 1935, **48**, 574.
9. Kleitman, N., Biological rhythms and cycles. Physiol. Rev., 1949, **29**, 1.
10. Borst, J. G. G., and de Vries, L. A., The three types of "natural" diuresis. Lancet, 1950, **2**, 1.
11. Sirota, J. H., Baldwin, D. S., and Villarreal, H., Diurnal variations of renal function in man. J. Clin. Invest., 1950, **29**, 187.
12. Mills, J. N., Diurnal rhythm in urine flow. J. Physiol., 1951, **113**, 528.
13. White, H. L., Rosen, I. T., Fischer, S. S., and Wood, G. H., The influence of posture on renal activity. Am. J. Physiol., 1926, **78**, 185.
14. Ni, T. G., and Rehberg, P. B., On the influence of posture on kidney function. J. Physiol., 1931, **71**, 331.
15. Brun, C., Knudsen, E. O. E., and Raaschou, F., Influence of posture on kidney function. I. The fall in the diuresis in the erect posture. Acta med. Scandinav., 1945, **122**, 315.
16. Kattus, A. A., Sinclair-Smith, B., Genest, J., and Newman, E. V., The effect of exercise on the renal mechanism of electrolyte excretion in normal subjects. Bull. Johns Hopkins Hosp., 1949, **84**, 344.
17. Strauss, M. B., Davis, R. K., Rosenbaum, J. D., and Rossmeisl, E. C., "Water diuresis" produced during recumbency by the intravenous infusion of isotonic saline solution. J. Clin. Invest., 1951, **30**, 862.
18. Simpson, G. E., The effect of sleep on urinary chlorides and pH. J. Biol. Chem., 1926, **67**, 505.
19. Stanbury, S. W., and Thomson, A. E., Diurnal variations in electrolyte excretion. Clin. Sci., 1951, **10**, 267.
20. Levy, M. S., Power, M. H., and Kepler, E. J., The specificity of the "water test" as a diagnostic procedure in Addison's disease. J. Clin. Endocrinol., 1946, **6**, 607.
21. Slessor, A., Studies concerning the mechanism of water retention in Addison's disease and in hypopituitarism. J. Clin. Endocrinol., 1951, **11**, 700.
22. Rosenbaum, J. D., Ferguson, B. C., Davis, R. K., and Rossmeisl, E. C., The influence of cortisone upon the diurnal rhythm of renal excretory function. J. Clin. Invest., in press.
23. Blomhert, G., Over de Zogenaamde Waterdiurese. Thesis. Amsterdam, 1951.
24. Rosenbaum, J. D., Nelson, W. P., III, and Strauss, M. B., The dependence of water diuresis upon electrolyte excretion. J. Clin. Invest., 1950, **29**, 841.
25. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Vol. II. Methods. Williams & Wilkins, Baltimore, 1932, pp. 832–835.
26. Peters, J. P., and Van Slyke, D. D., *Ibid.*, pp. 838–839.
27. Van Slyke, D. D., and Hiller, A., Application of Sendroy's iodometric chloride titration to protein-containing fluids. J. Biol. Chem., 1947, **167**, 107.
28. Peters, J. H., The determination of creatinine and creatine in the blood and urine with the photoelectric colorimeter. J. Biol. Chem., 1942, **146**, 179.
29. Hare, R. S., Endogenous creatinine in serum and urine. Proc. Soc. Exper. Biol. & Med., 1950, **74**, 148.
30. Burn, G. P., and Grewal, R. S., The antidiuretic response to and excretion of pituitary (posterior lobe) extract in man, with reference to the action of nicotine. Brit. J. Pharmacol., 1951, **6**, 471.
31. Strauss, M. B., Davis, R. K., Rosenbaum, J. D., and Rossmeisl, E. C., The production of increased renal sodium excretion by the hypotonic expansion of extracellular fluid volume in recumbent subjects. J. Clin. Invest., 1952, **31**, 80.

POTASSIUM AND SODIUM OF RED BLOOD CELLS IN SICKLE CELL ANEMIA[1]

By D. C. TOSTESON, ETHEL SHEA, AND R. C. DARLING

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University; and the Presbyterian Hospital in the City of New York)

(Submitted for publication December 15, 1951; accepted February 5, 1952)

Normal human red cells contain approximately 100 mEq./l. of potassium (K) and 10 mEq./l. of sodium (Na). The K composition of sickle cell anemia (hereinafter designated SCA) red cells was first investigated by Erickson and her co-workers (1) who found that red cell [K] in SCA was approximately 10% less than normal red cell [K].

Our interest in the distribution of cations in SCA blood was stimulated by the observation of elevated venous serum [K] in three patients with SCA. Erickson and her co-workers did not report what effect varying blood oxygen content had on [K] and [Na] in SCA red cells. Since oxygen is known to affect other properties of SCA red cells (*2, 3*), it was decided to investigate the effect of controlling this variable. Therefore measurements of SCA red cell [Na] and [K] were made in both oxygenated and de-oxygenated states, *in vivo* and *in vitro*.

METHODS

1. Analytical techniques

K and Na were measured with a barrier layer cell flame photometer operated with a lithium internal standard (4). It is estimated that these measurements are accurate to ± 2%. When measured, water content of the red cells was determined by weighing a sample before and after drying in an oven at 110° C. for 24 or more hours. In some instances, pO_2 and pCO_2 in the gas phase of the experiments were measured by the method of Scholander (5), and the O_2 and CO_2 content of the blood phase by the technique of Van Slyke and Neill (6). The red cells for K and Na analyses were pipetted by syringe suction with an Ostwald pipette calibrated to contain the desired volume.

2. Experimental procedures

(*a*) *Short term experiments in vitro.* Forty to fifty ml. of blood were drawn from the antecubital vein of normal subjects and patients with SCA. The blood was immediately heparinized with approximately 50 cat units of crystalline heparin per 10 ml. blood. Within one-half to one hour, 15-20 ml. of this heparinized venous blood were placed in each of two Barcroft tonometers (300 ml. capacity), one containing 30%O_2–5%CO_2–65%N_2, and the other containing 0%O_2–5%CO_2–95%N_2. The tonometers were sealed with Hg and rotated at 20 rpm. in a water bath at 37° C. for 20–30 minutes. The blood was then removed under oil and, in some instances, whole blood samples taken for gas analysis. The remainder of the blood was centrifuged under oil at 14×10^3 g. for one hour, after which samples of plasma and red cells were taken for K, Na, and water analysis as indicated.

In order to test the reversibility of the cation changes observed in the experiments described above, a 60 ml. sample of fresh heparinized venous SCA blood was first deoxygenated by equilibration for one-half hour in a Barcroft tonometer at 37° C. with 95%N_2–5%CO_2, as before. Then a 20 ml. sample was removed for analysis, the gas phase was flushed out with 95%O_2–5%CO_2 and the remaining 40 ml. of blood re-equilibrated for one-half hour with this gas. Next, a second sample was taken out, the final 20 ml. of blood were again deoxygenated by equilibrium in 95%N_2–5%CO_2. This blood was then the third sample. All blood samples were collected and centrifuged under oil at 800 g. for five minutes and plasma samples taken. The remaining loosely packed red cells were then centrifuged at 14×10^3 g. as before, the remaining plasma removed, and red cell samples taken for measurement of Na, K, and water content.

Since carbon monoxide prevents the occurrence of sickling of SCA red cells in a hypoxic medium (*2*), it was decided to utilize this fact to study the effect of hypoxia on cation changes in the absence of sickling. Forty ml. of fresh heparinized venous SCA or normal blood were gently shaken in a flask containing approximately 25%CO–72%N_2–3%CO_2 until cherry red in color. Twenty ml. samples of this blood were then placed in each of two Barcroft tonometers, one containing 95%O_2–5%CO_2, the other containing 95%N_2–5%CO_2. The tonometers were then sealed and rotated at 37° C. for one-half hour, after which samples were taken under oil and prepared for analysis as before.

(*b*) *Long term in vitro experiments.* In order to investigate changes in SCA red cell [K] over a longer period of time, a different technique was employed for several reasons: *1*) SCA red cells kept in the de-oxygenated state for several hours become extremely viscous and

[1] This research was aided by a grant to Columbia University from the Baruch Committee on Physical Medicine and Rehabilitation.

TABLE I

Results of typical experiments on one normal subject and one patient with SCA

Each blood sample was incubated for one-half hour in the oxygenated and de-oxygenated state.

Subject	Gas		Blood gas		Red blood cell					Plasma			
	pO_2	pCO_2	O_2	CO_2	Na	K	Na+K	H_2O	pH	Na	K	Na+K	pH
	mm.Hg	*mm.Hg*	*vol. %*	*vol. %*	*mEq./l.*	*mEq./l.*	*mEq./l.*	*%*	*calc.*	*mEq./l.*	*mEq./l.*	*mEq./l.*	*calc.*
Normal	215	45	22.8	46	11	98	109	66.0	7.19	136	3.7	140	7.37
	12	42	3.4	50	11	96	107	66.0	7.25	136	3.7	140	7.44
Sickle cell anemia	237	42	8.5	50	27	83	110	64.1	7.19	134	4.5	139	7.37
	8	41	0.4	51	40	60	100	63.4	7.19	133	7.0	140	7.38

difficult to pipette, *2*) heparinized SCA blood in a Barcroft tonometer rotated at 37° C. for 24 hours shows considerable hemolysis, *3*) the vigorous centrifugation used to separate red cells and plasma might conceivably affect the results.

Forty ml. of fresh heparinized venous SCA blood were placed in a flask containing 60 ml. of a bicarbonate-phosphate buffered electrolyte solution, (pH 7.4; [K] = 4.4 mEq./l.; [Na] = 135 mEq./l.) containing 4.0 gm./l. of glucose. This solution is known to maintain normal human red cells in a relatively stable state for 48 hours (*7*). In our experiments visible hemolysis was present in only one sample and then was barely detectable by the naked eye. Twenty-five ml. of the resultant diluted blood were placed in each of four Parafilm sealed Erlenmeyer flasks with open side-arms to allow gas flow. Ninety-five%O_2–5%CO_2 was flushed through the flasks which were then rocked 12 × /min. through an arc of 30–40° in a water bath at 37° C. for one-half hour. One flask was then taken from the bath and the diluted blood removed under oil. Either 95%O_2–5%CO_2 or 95%N_2–5%CO_2 was flushed through the remaining three flasks which were then rocked in the bath as before. Samples of diluted blood were taken under oil at four, 12, and 24 hours. Hematocrit was determined on all samples by centrifugation at 800 g. for one hour. K in diluted blood and diluted plasma was measured by flame photometer, as before. Red cell [K] was calculated from these data by the following formula: $K_c = 1/\gamma\ K_b - K_p\ (1-\gamma)$ when K_c = red cell [K], K_b = diluted blood [K], K_p = diluted plasma [K], and γ = hematocrit corrected for incomplete packing (*8*).

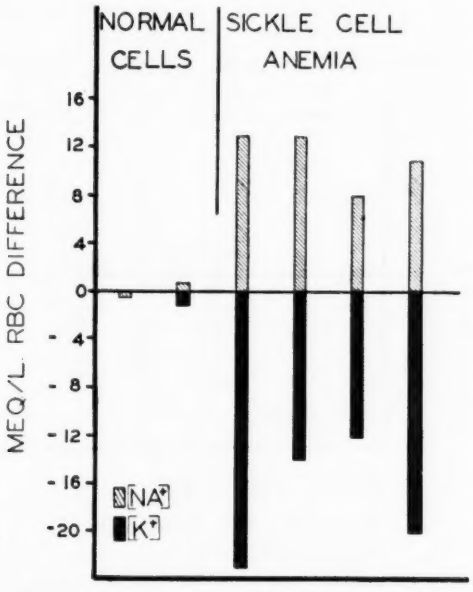


FIG. 1. EXPERIMENTS ON TWO NORMAL SUBJECTS AND FOUR PATIENTS WITH SCA—EACH BLOOD SAMPLE INCUBATED FOR ONE-HALF HOUR IN THE OXYGENATED AND DE-OXYGENATED STATE

Each bar represents the difference between oxygenated and de-oxygenated red cell sodium or potassium concentration.

(*c*) *In vivo experiments.* In order to investigate the initial phase of the cation changes *in vivo*, 20 ml. samples of blood were drawn simultaneously, under oil, from the antecubital vein and opposite brachial or femoral artery. Drawing of the venous sample was preceded by five to 30 minutes of venous congestion produced by a blood pressure cuff at 90 mm. Hg. The samples were immediately heparinized and centrifuged under oil at 14×10^3 g. for one hour. Samples of red cells and plasma were analyzed for K, Na, and water as before.

RESULTS

(*a*) *Short-term experiments in vitro*

In one experiment (Table I) typical of four with SCA red cells, [K] was about 23 mEq./l. RBC less and [Na] about 13 mEq./l. RBC more, in the de-oxygenated than in the oxygenated state. This loss of K and gain of Na was not evident upon de-oxygenation of normal cells. Even in the oxygenated state, SCA red cell [K] was somewhat less and [Na] somewhat more than the values in nor-

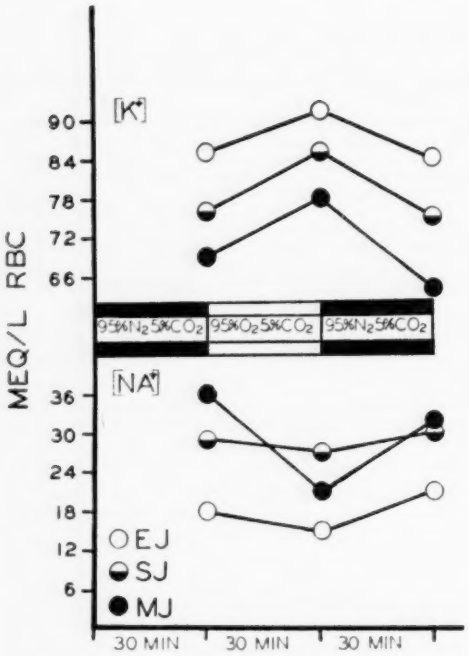


FIG. 2. EXPERIMENTS ON THREE PATIENTS WITH SCA —EACH BLOOD SAMPLE INCUBATED CONSECUTIVELY FOR ONE-HALF HOUR IN THE DE-OXYGENATED, OXYGENATED, AND DE-OXYGENATED STATE

mal red cells. Since the loss of K exceeded the gain of Na by de-oxygenated SCA red cells, the sum of [K] + [Na] was slightly less in the de-oxygenated than in the oxygenated state.

The water content of SCA red cells was slightly less than the value for normal red cells in the oxygenated state. De-oxygenation resulted in a slight fall in SCA red cell water content but did not affect normal red cell water content. Since the water content of de-oxygenated SCA red cells was, if anything, slightly less than the oxygenated value, it is unlikely that increased plasma contamination due to incomplete packing of the sickled de-oxygenated cells caused the observed reduced [K] and increased [Na] in these cells. pH as calculated by the method of Henderson (9) was essentially the same in normal and SCA red cells in oxygenated and de-oxygenated states. The Donnan r value for H^+ was assumed to be the same for normal and SCA blood (10).

Figure 1 presents a graphic summary of data obtained from four SCA patients and two normal subjects. The results qualitatively confirmed all the findings described above for a single experiment. The blood of every SCA patient studied showed significantly less red cell K and more Na in the de-oxygenated than in the oxygenated state. These differences were not present in eight experiments on two normal subjects.

Figure 2 presents data which demonstrate the reversibility of the K loss and Na gain of previously de-oxygenated SCA red cells upon re-oxygenation. In experiments on three SCA patients, the mean rates of K gain by re-oxygenated and K loss by de-oxygenated red cells were both about 20 mEq./l. RBC/hr. The mean rates of Na loss by re-oxygenated and gain by de-oxygenated red cells were both about 14 mEq./l. RBC/hr.

Figure 3 shows that CO blocked the K loss and Na gain by de-oxygenated SCA red cells. CO did not affect the cation concentrations of normal red

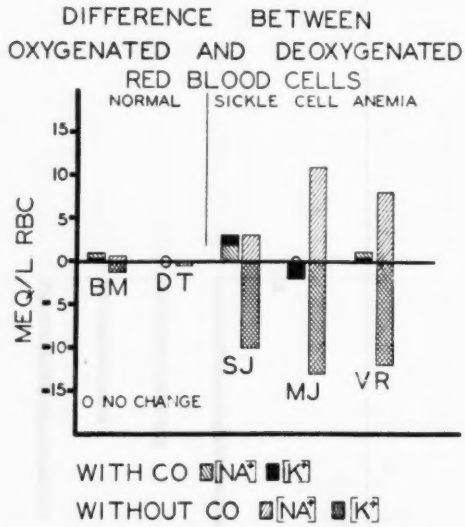


FIG. 3. EXPERIMENTS ON TWO NORMAL SUBJECTS AND THREE PATIENTS WITH SCA—EACH BLOOD SAMPLE INCUBATED FOR ONE-HALF HOUR IN 95% O_2 AND 95% N_2, WITH AND WITHOUT PRE-EXPOSURE TO 25% CO

The bar on the left of each pair represents the difference between oxygenated and de-oxygenated red cell sodium or potassium concentration with pre-exposure to CO. The bar on the right of each pair represents the same quantity without pre-exposure to CO.

cells. Thus, hypoxia *per se* does not cause the K-Na exchange observed in de-oxygenated SCA red cells.

(b) Long-term experiments in vitro

In the experiments with slow gentle shaking (see Methods) de-oxygenated SCA red cells continued to lose K for at least 24 hours (Figure 4). K loss from oxygenated SCA red cells was small and irregular in the two cases studied. The rate of K loss during the first four hours from de-oxygenated SCA red cells in this system was about 4 mEq./l. RBC/hr. The rate of loss decreased with time but was still appreciable between 12 and 24 hours after the start of the experiment. In a system identical with that employed here Raker and his associates (7) working with normal cells did not observe accelerated K loss upon de-oxygenation.

(c) Short-term experiments in vivo

Figure 5 shows the cation difference in four SCA patients between red cells from congested

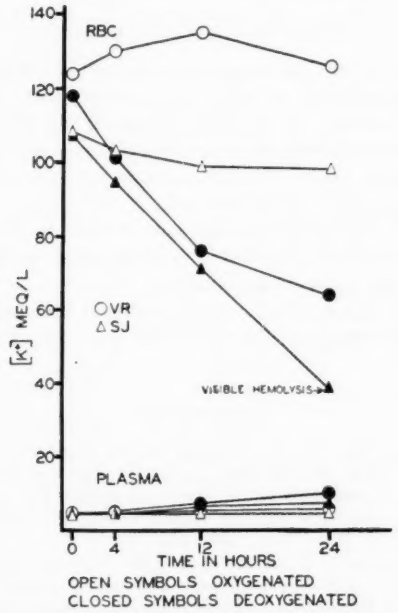


FIG. 4. EXPERIMENTS ON TWO PATIENTS WITH SCA—EACH SAMPLE OF DILUTED BLOOD INCUBATED FOR 24 HOURS IN THE OXYGENATED AND DE-OXYGENATED STATE

The de-oxygenated 24-hour sample of SJ showed hemolysis just detectable by the naked eye.

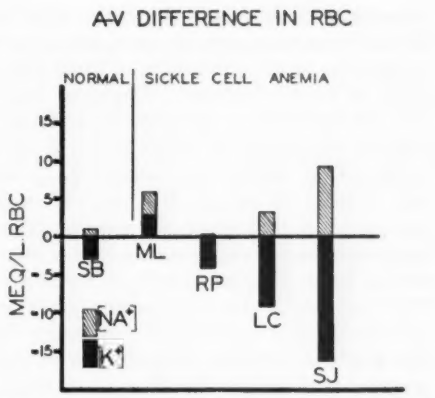


FIG. 5. EXPERIMENTS ON ONE NORMAL SUBJECT AND FOUR PATIENTS WITH SCA—EACH BAR REPRESENTING THE ARTERIOVENOUS DIFFERENCE IN RED CELL NA OR K CONCENTRATION

veins and arterial red cells. In general, venous red cell [K] was less and the [Na] more than the arterial values. The A–V differences varied from a maximum of − 17 mEq. K/l. RBC and + 9 mEq. Na/l. RBC in one patient to a minimum of + 3 mEq. K/l. RBC and 0 mEq. Na/l. RBC in another. There was no A–V difference in plasma [K] so that we were unable to produce experimentally the clinically observed elevation of venous plasma [K]. In the normal subject, A–V differences in red cell and plasma [K] and [Na] were small.

DISCUSSION

Human red cells are known to lose K and gain Na on exposure to cold (11), X-rays (12), iodoacetate (13) and fluoride (13–15), in the absence of glucose (14, 15), and possibly upon infestation with malarial parasites (16). Ponder describes red cell K loss and Na uptake prior to hemolysis in a variety of *in vitro* hemolytic systems (17). The mechanism of such "prolytic" K–Na exchange is obscure. The electrolyte shift which we found during hypoxia is, in our experience, unique to SCA and possibly sickle cell trait red cells. Whether this process is also "prolytic" is beyond the scope of this paper.

The initial rate of K loss from SCA red cells de-oxygenated in a Barcroft tonometer (about 20 mEq./l. RBC/hr.) was considerably faster than the value for SCA red cells de-oxygenated in an

Erlenmeyer flask (about 4 mEq./l. RBC/hr.). This difference was perhaps due to the slower rate of agitation or of equilibration of blood with gas phase in the latter system. During at least the first one-half hour of de-oxygenation the K loss could be reversed by re-oxygenation. In this circumstance, K uptake also occurred at an initial rate of about 20 mEq./l. RBC/hr. Since these rates of net K uptake and loss were considerably faster than the rate of turnover of K in normal red cells in the steady state (2 mEq./l. RBC/hr.) (7, 18), it is probable that under certain conditions K turnover in SCA red cells is considerably faster than in normal red cells. Experiments are now in progress to determine the effect of variations in temperature, concentration gradient, etc. on the rates of net K and Na transfer and to measure with appropriate isotopes the rate of turnover of these ions in SCA red cells.

Why do these electrolyte changes occur in de-oxygenated SCA red cells but not in normal cells? Two general hypotheses are plausible:

(*1*) K–Na exchange is due to an abnormality in SCA plasma. For example, "prolytic" ion exchange as described by Ponder is particularly large in hemolytic systems containing bile salts. Most SCA patients were slightly jaundiced but accurate determinations were not made of the concentration of bile salts in their plasma. It does not seem possible that plasma bile salts *per se* cause the cation exchange observed in SCA red cells because (*a*) it is unlikely that reduction in pO_2 would accelerate such a process, and (*b*) red cells taken from a patient with severe obstructive jaundice due to carcinoma of the head of the pancreas did not lose K or gain Na during 24 hours of incubation at 37° C. in the oxygenated or de-oxygenated state. Since the shift occurred in de-oxygenated SCA red cells suspended in diluted plasma and (in one experiment) Ringer's solution, evidence for a plasma abnormality responsible for the process is at present lacking.

(*2*) K–Na exchange is due to an abnormality in SCA red cells. The most obvious difference between the responses of normal and SCA red cells to hypoxia is the occurrence of sickling in the latter. Several facts suggest that K loss and Na uptake by de-oxygenated SCA red cells is intimately associated with the process of sickling: (*a*) CO prevents both sickling and K–Na exchange in de-oxygenated SCA red cells, (*b*) both processes begin almost immediately after de-oxygenation of SCA blood.

What is the mechanism relating the processes of sickling and K–Na exchange in SCA red cells? The change of shape and probable mechanical distortion of the red cell membrane in the sickled state are obvious. These changes could accelerate the diffusion of K and Na in the direction of their concentration gradients. In addition, recently demonstrated differences between ionic (19), and physical (3, 12, 20) properties of SCA and normal hemoglobin may be related to cation exchange in sickled SCA red cells. Specifically, the work of Harris (3) and of Perutz and Mitchison (20) suggests that unsaturated SCA hemoglobin is in a crystalline or para-crystalline state. It is not yet clear whether the observed changes in cation composition of *sickled* SCA red cells are primarily related to the physical properties of desaturated SCA ***hemoglobin*** or to alteration of the SCA red cell ***membrane*** incident to the sickle shape.

SUMMARY AND CONCLUSIONS

1. In the de-oxygenated but not in the oxygenated state, red cells from patients with sickle cell anemia (SCA) begin to lose K and gain Na almost immediately and continue to do so for at least 24 hours. This process does not occur in de-oxygenated normal red cells.

2. During the first half hour at least, K–Na exchange in de-oxygenated SCA red cells can be reversed by re-oxygenation.

3. K loss and Na gain by red cells does not occur in the absence of O_2 if sickling is prevented by CO.

REFERENCES

1. Erickson, B. N., Williams, H. H., Hummel, F. C., Lee, P., and Macy, I. G., The lipid and mineral distribution of the serum and erythrocytes in the hemolytic and hypochromic anemias of childhood. J. Biol. Chem., 1937, **118**, 569.
2. Hahn, E. V., and Gillespie, E. B., Sickle cell anemia: report of a case greatly improved by splenectomy. Experimental study of sickle cell formation. Arch. Int. Med., 1927, **39**, 233.
3. Harris, J. W., Studies on the destruction of red blood cells. VIII. Molecular orientation in sickle cell hemoglobin solutions. Proc. Soc. Exper. Biol. & Med., 1950, **75**, 197.

4. Berry, J. W., Chapell, D. G., and Barnes, R. B., Improved method of flame photometry. Indust. & Engin. Chem. (Analyt Ed.), 1946, **18**, 19.
5. Scholander, P. F., Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. J. Biol. Chem., 1947, **167**, 235.
6. Van Slyke, D. D., and Neill, J. M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. J. Biol. Chem., 1924, **61**, 523.
7. Raker, J. W., Taylor, I. M., Weller, J. M., and Hastings, A. B., Rate of potassium exchange of the human erythrocyte. J. Gen. Physiol., 1950, **33**, 691.
8. Millar, W. G., Observations on the haematocrite method of measuring the volume of erythrocytes. Quart. J. Exper. Physiol., 1925, **15**, 187.
9. Henderson, L. J., Blood, a Study in General Physiology. Yale Univ. Press, New Haven, 1928.
10. Dill, D. B., Edwards, H. T., and Consolazio, W. V., Blood as a physico-chemical system. XI. Man at rest. J. Biol. Chem., 1937, **118**, 635.
11. Smith, M. E., Tuthill, E., Drew, C. R., and Scudder, J., Studies in blood preservation. Some effects of carbon dioxide. J. Biol. Chem., 1940, **133**, 499.
12. Sheppard, C. W., and Beyl, G. E., Cation exchange in mammalian erythrocytes. III. The prolytic effect of x-rays on human cells. J. Gen. Physiol., 1951, **34**, 691.
13. Wilbrandt, W., A relation between the permeability of the red cell and its metabolism. Tr. Faraday Soc., 1937, **33**, 956.
14. Harris, J. E., The influence of the metabolism of human erythrocytes on their potassium content. J. Biol. Chem., 1941, **141**, 579.
15. Danowski, T. S., The transfer of potassium across the human blood cell membrane. J. Biol. Chem., 1941, **139**, 693.
16. Overman, R. R., Hill, T. S., and Wong, Y. T., Physiological studies in the human malarial host. I. Blood, plasma, extracellular fluid volumes and ionic balance in therapeutic *P. vivax* and *P. falciparum* infections. J. Nat. Malaria Soc., 1949, **8**, 14.
17. Ponder, E., Hemolysis and Related Phenomena. Grune & Stratton, New York, 1948.
18. Sheppard, C. W., and Martin, W. R., Cation exchange between cells and plasma of mammalian blood. I. Methods and application to potassium exchange in human blood. J. Gen. Physiol., 1950, **33**, 703.
19. Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C., Sickle cell anemia, a molecular disease. Science, 1949, **110**, 543.
20. Perutz, M. F., and Mitchison, J. M., State of haemoglobin in sickle-cell anaemia. Nature, 1950, **166**, 677.

STUDIES OF TOTAL BODY WATER WITH TRITIUM [1, 2]

By T. C. PRENTICE, W. SIRI, N. I. BERLIN, G. M. HYDE, R. J. PARSONS, E. E. JOINER, AND J. H. LAWRENCE

(From the Section on Experimental Medicine, Donner Laboratory, University of California, Berkeley, and the Highland Alameda County Hospital, Oakland, Cal.)

(Submitted for publication November 5, 1951; accepted February 11, 1952)

The precise measurement of total body water in man by *in vivo* methods is becoming increasingly important as more is learned of the significance of electrolyte and water metabolism in normal and pathologic states. While there is as yet insufficient evidence to suggest that body water determinations *per se* will prove of significant value in diagnosis and therapy, the investigation of body water appears essential to a fuller understanding of disease states in which electrolyte and water balance is altered. Thus far, such measurements remain largely laboratory experiments with the bulk of the data obtained from normal subjects. However, with the present improvements in the methods for assaying tritium, either with the ionization chamber (1) or the proportional counter (*2*), the study of total body water and water metabolism by tracer methods will doubtless be greatly accelerated.

The present study was undertaken to develop further the use of tritium for routine body water measurement and to extend the investigation of total body water to a variety of disease states. A rapid method for routine assay of tritium had already been developed in this laboratory, but there still remained several factors which, *a priori*, could influence the accuracy of total body water determinations with tritium labeled water. The time required for complete mixing throughout the body water following intravenous administration of tritium labeled water was considered particularly important in selecting the time at which blood samples could be taken to determine the amount of dilution. It was also of special interest to investigate the possible existence of a significant isotope effect as well as to evaluate by more direct methods, the magnitude of the error introduced by exchange of tritium with the solid constituents of the body.

Total body water has been measured by many investigators using four principal methods: *1*) post-mortem desiccation of cadavers (3), *2*) balance studies of electrolytes and water (4), *3*) determination of the specific gravity of the whole body *in vivo* (5, 6) and *4*) the dilution of various substances presumed to be limited to and uniformly mixed within the body water. Well documented reviews of the literature have recently appeared (*5, 7*).

Method *1*) has no practical significance in clinical investigation. Method *2*) requires painstaking balance experiments and no claim is made for quantitative accuracy. Method *3*), the measurement of whole body specific gravity, has received considerable recent attention. It is based on the premise that the body can be divided into two portions, namely, the fat-free portion, called the lean body mass, and adipose tissue. The lean body mass purportedly maintains a relatively constant gross composition, thus contributing a constant known effect to whole body specific gravity. The fat content, on the contrary, varies widely from individual to individual and is normally the largest factor responsible for the variation of body density. This basic premise has been substantiated by careful work in guinea pigs and rats (5, 8). In large groups of these animals, whole body specific gravity was measured and thereafter total body fat determined gravimetrically. A hyperbolic relationship between specific gravity and body fat was found which agreed closely with the curve predicted from theoretical considerations (9). The equation for this curve in humans, $\%\ \text{fat} = 100 \times \left(\frac{5.548}{\text{sp. gr.}} - 5.044\right)$, provides a reasonably accurate estimate of total body fat when the specific gravity

[1] This work was supported by the Life Insurance Medical Research Fund and the U. S. Atomic Energy Commission.

[2] We are grateful to Dr. Leo Stanley, of the California State Prison at San Quentin, and to volunteers from the prison who served as subjects for the normal, middle-aged male group.

can be measured. Since water constitutes, on the average, 73% of the fraction remaining after subtracting fat, then % body water = 0.73 (100 − % fat). The validity of this method in clinical investigation should be questioned from two standpoints. First, since all the experimental work was done in guinea pigs and rats, can the results be extrapolated to man? Comparisons of total body water estimated by this method with values obtained using tritium (10) or antipyrine (6) agree closely on the average in normal man. Second, is the method valid for estimating total body water during disease states? Experimental work is lacking on this point, but the marked changes which frequently occur both in the distribution and in the amounts of many body constituents during disease, coupled with the dependency of this method on constant composition of the lean body mass, make it unlikely that accurate results could be obtained using this method alone.

The most direct, and potentially the most accurate method of measuring total body water is the dilution method. Many substances have been tested, but most have proved unsatisfactory because of failure to limit themselves to and distribute themselves equally throughout body water. Deuterium (11), tritium (12), and antipyrine (13, 14) have been used most successfully thus far. Aside from differences in technical difficulties, which for deuterium are often considerable, deuterium and tritium are equivalent. Both give body water volumes which appear to be slightly greater than the true volume because of exchange with hydrogen in protein. Antipyrine, on the other hand, requires serial sampling in order to compensate for its relatively rapid disappearance from plasma.

METHODS

A total of 57 subjects, including both normals and those with disease states, were used in the present study. Thirty-seven of the subjects were patients with cardiac, liver, and blood abnormalities. Normal subjects were selected primarily in the middle age range inasmuch as previous studies were made predominantly in relatively young, active subjects. Among the normal subjects, 15 were in the age group of 34 to 56 years. Four of the five remaining normal subjects were 20 to 25 years of age.

The tritiated water used throughout the study was prepared as a stock solution with a specific activity of approximately 2 mc./cc. One cc. of the sterilized stock solution was given intravenously with a tuberculin syringe. It should be noted that the use of tuberculin syringes for this purpose has sometimes been criticized on the grounds that they are not sufficiently reliable for delivering an accurately known volume of fluid, particularly when the same syringe is not used throughout a series of injections. This criticism was tested in the present instance by gravimetric determination of the volume of water delivered by tuberculin syringes selected at random. Although the test cannot be regarded as exhaustive, the syringes tested showed a maximum error of no more than 2% in the 1 cc. volume delivered, while the variation in volume delivered from any single syringe did not exceed 0.5%. This result is in agreement with similar tests made in this laboratory to assess the error in tuberculin syringes used for blood volume determination with P^{32} labeled red cells.

Following administration of the tritiated water, heparinized blood samples were withdrawn at intervals of two, four, and six hours. The plasma was separated by centrifugation, placed in sealed tubes and frozen until analyzed. At two hours, mixing appeared in every case to be complete, and if differences in plasma tritium concentration did exist at the two later intervals, such changes were within the experimental error; consequently, the values of the specific activities of the three plasma samples were averaged. An earlier practice of taking samples at three, six, and 24 hours, with the intention of extrapolating the specific activity of plasma to the time of injection was abandoned in patients without fluid retention in view of the relatively long turnover time of about ten days for water in humans (12). Nevertheless in all patients with edema or any collection of fluid in the serous cavities the three, six and 24 hour time periods for sampling were used. It was felt that averaging the two, four, and six hour samples was more reliable than the extrapolation from samples taken over the longer intervals in patients in whom fluid retention was not present.

The tritium activity was assayed with 100 cc. cylindrical ionization chambers which were filled to atmospheric pressure with hydrogen generated from 0.5 cc. of plasma sample. Reduction of the water was effected *in vacuo* with lithium aluminum hydride dissolved in diethyl carbitol (1-ethoxy-2-[B-ethoxyethoxy] ethane) as described by Siri. The rate of ionization was measured with a vibrating reed electrometer and recording potentiometer.

A tritium standard, prepared by diluting a portion of the stock solution by a factor of 20,000, was run in each ionization chamber at frequent intervals. Total body water volumes were computed from the simple dilution formula: 20 × specific activity of standard/specific activity of plasma = total body water in liters. These are the volumes given in Tables II to V inclusive, and do not include the small correction for hydrogen exchange discussed later because the correction is that for an idealized "average" man. The correction would vary somewhat with each individual, depending upon the ratio of water to lean tissue.

Antipyrine, used for body water volume comparisons, was prepared as a sterile solution of 0.9 gm. antipyrine in 45 cc. water and the whole given intravenously. Heparinized blood samples were taken at the same time as those for the tritium assay, namely, at two, four, and six hours. Plasma antipyrine levels were determined by the method described by Brodie, Axelrod, Soberman, and Levy (13), using a Beckman DU spectrophotometer. The plasma concentration of antipyrine, in contrast with that of tritiated water, exhibits a relatively rapid exponential disappearance with an average half time of about 11 hours; consequently, the correct dilution factor must be found by extrapolating the plasma concentration curve back to the time of injection.

The exchange of tritium with hydrogen in tissue solids was determined from the uptake of tritium in a representative selection of organs of rabbits. Each of four rabbits was injected with 0.1 mc. of tritium and exsanguinated after three hours. Plasma was assayed directly for tritium, while samples of plasma solids, muscle, liver, kidney, G.I. tract, and fat were first dried *in vacuo* to constant weight before analysis. For tritium assay, the dried solids were oxidized to water in a stream of oxygen in a quartz tube and passed over copper oxide at 850° C. The water collected in a CO_2 trap was then analysed for tritium as before.

OBSERVATIONS

Arterial mixing of tritiated water, investigated in this laboratory by Pace and associate (15), reveals that mixing is essentially complete in normal adults well within two hours. In the present investigation, early changes in the specific activity of tritium in plasma were studied serially in two patients with marked ascites in whom mixing might be expected to be greatly altered. However, as shown in Figure 1, after two hours, mixing appears to be complete, at least within the accuracy of measurement, in both these individuals. Subsequent studies in patients with fluid retention indicate that long half-time mixing components are present. However, in the present studies the 24 hour sample taken in patients with fluid retention minimizes any error due to slow mixing components.

In simple dilution methods such as those used here, where equilibrium is first established, it is highly unlikely that even the great mass difference between hydrogen and tritium can introduce an isotope effect of consequence. However, without direct comparison with deuterium, this is difficult to demonstrate. The comparison, nevertheless, could be made indirectly with the results of studies in which antipyrine and deuterium water volumes were investigated. If no isotope effects were present, the ratios (H^3 body water)/(antipyrine body water) and (H^2 body water)/(antipyrine body water) should be equal. This was, in fact, observed on comparing the present tritium-antipyrine body water volumes with the deuterium-antipyrine body water volume reported by Soberman and associates (14). At least within the present experimental error, an isotope effect does not appear to be a measurable factor.

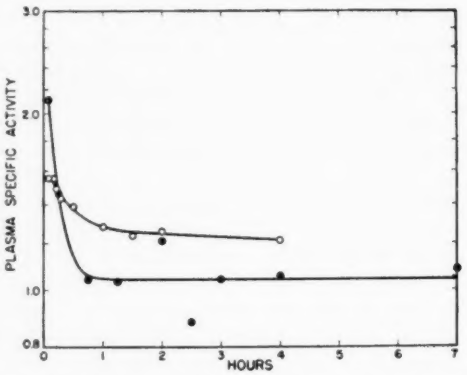


FIG. 1. TRITIUM CONCENTRATION IN VENOUS BLOOD AS A FUNCTION OF TIME IN TWO PATIENTS WITH CIRRHOSIS OF THE LIVER AND ASCITES

Plasma specific activity is expressed in units of activity relative to the tritium standard.

Previous investigators (14) have observed that on the average, a small but relatively constant difference in total body water is found with deuterium as compared to antipyrine. As indicated above, a similar difference between tritium and antipyrine water volumes is found in which the tritium dilution volume is slightly greater than that for antipyrine. This difference can be accounted for by exchange of tritium with labile hydrogen atoms of proteins and carbohydrates. The body water (plasma) specific activity is consequently lowered, giving the appearance of larger volume. As shown in Table I, the specific activity of tritium in various organs of the rabbit varied from 6% to 15% of that for body water. This agrees closely with the observations of Ussing with deuterium (16). Assuming that exchange of the same order of magnitude as in rabbits also takes place in the corresponding tissue of man, a correction of about 2% in total body water can be calculated, which is in agree-

TABLE I

Exchange of tritium in tissues relative to body water

Except for plasma, all tissues were first dried to constant weight before analysis for tritium. The values given in the table are adjusted to a specific activity of 100 for plasma (body water), and are expressed as activity (in arbitrary units) per gram of tissue or plasma.

Organ	Rabbit			
	I	II	III	IV
Plasma	100.0	100.0	100.0	100.0
Liver	14.0	10.0	15.4	
Kidney	13.4	8.9	13.6	
G. I. tract	8.0	7.5	11.9	
Muscle	8.0	5.9	11.8	
Plasma solids	15.4	15.0		15.2
Fat	—	—	0	—

ment with estimates of 0.5% to 2.0% by other investigators (11).

The rabbit data in Table I indicate a difference in lability of hydrogen atoms in various tissue masses of the body. No exchange could be detected in fat. Liver, kidney, and plasma solids show a consistently higher percentage of exchange than do muscle and G.I. tract. This division corresponds more or less with viscera and carcass, and for the purpose of estimating a correction for hydrogen exchange, no large error is introduced by considering the entire viscera as high activity tissue and carcass, generally, as representing the less labile tissue. An average value of 64% body weight was taken for total carcass, 24% for total viscera and 12% was considered fat in a "standard" man. Since adipose tissue contains little water, the true body water should equal 73% of the lean body mass. The observed body water is greater than this by the water equivalent of the tritium which exchanges in protein (carbohydrates are not considered here because they constitute but a small fraction of the total organic constituents). Thus, observed body water per cent = 73% + relative specific activity of carcass proteins × per cent of body weight + relative specific activity of viscera × per cent of body weight.

TABLE II

Comparison of total body water obtained with tritium and antipyrine in patients and normals

Subject	Age	Sex	H^3 body water (*liters*)	H^3 body water (%)	Antipyrine body water (*liters*)	Antipyrine body water (%)	Diagnosis
A. Patients							
P. C.	64	M	35.2	55.3	33.1	52.0	Poly. vera
L. Y.	66	F	26.8	50.0	25.3	47.2	Poly. vera
T. F.	63	M	40.6	67.1	39.4	65.2	Poly. vera
B. L.	76	M	35.6	51.0	33.7	48.4	Poly. vera
S. B.	59	F	30.0	50.0	29.0	48.4	Lymph. leuk.
B. A.	63	M	49.6	65.1	48.8	64.1	Lymph. leuk.
T. C.	40	M	47.6	65.7	50.0	68.9	Myel. leuk.
D. A.	63	M	44.0	48.8	42.6	46.8	Sec. poly.
M. B.	46	F	28.2	59.6	26.5	56.1	Congest. fail.
B. A.	50	F	20.2	49.3	20.1	48.8	Cirrhosis
Mean	59.0		35.8	56.2	34.8	54.6	
S. D.				± 7.5		± 8.7	
B. Normal subjects							
J. M.	22	M	41.5	60.5	39.4	57.4	
R. N.	25	M	43.4	57.5	39.5	52.4	
P. K.	22	M	55.0	60.4	51.2	56.2	
R. C.	20	M	48.6	63.0	47.4	61.4	
C. G.	55	M	40.6	53.3	40.5	53.1	
Mean	28.8		45.8	58.9	43.6	56.1	
S.D.				± 3.7		± 3.6	

TABLE III

Total body water of normal male subjects

Total body water was measured with tritium labeled water.

Subject	Age	Height	Weight (*Kg.*)	Body water (*liters*)	Body water (%)
1	44	5′6″	54.1	29.7	54.7
2	40	6′	91.4	43.9	48.1
3	54	5′11″	70.5	35.2	50.0
4	42	5′10″	91.0	46.8	51.4
5	38	5′8″	77.8	40.8	52.6
6	41	5′7″	68.2	38.7	56.7
7	42	5′8″	84.0	44.4	52.7
8	47	6′	75.5	38.5	50.6
9	53	5′7″	59.5	33.9	56.7
10	34	5′5″	67.5	34.9	51.7
11	41	4′8″	75.5	37.1	49.1
12	53	6′1″	88.6	46.9	52.7
13	45	5′8″	73.1	36.6	49.9
14	37	5′10″	78.2	44.2	56.5
15	56	5′11″	86.0	40.6	47.9
Mean	44.5	5′8″	76.0	39.5	52.1
S.D.					± 3.0

$$0.08 \times 0.795 \times 0.27 \times 56.5 = 0.96\% \text{ of body weight,}$$

while the third term is:

$$0.13 \times 0.795 \times 0.27 \times 24 = 0.67\% \text{ of body weight.}$$

Where 0.08 and 0.13 are the relative specific activities for carcass and viscera, 0.795 is the fraction of protein in dry, fat-free tissue found by

TABLE IV

Total body water of cardiac patients with and without failure

All body water determinations were made with tritium labeled water.

Patient	Age	Sex	Weight (*Kg.*)	Body water (*liters*)	Body water (%)	Diagnosis
A. Compensated at bed rest						
M. K. (2)	34	F	46.0	28.0	61.0	H.C.V.D.
B. F.	47	M	70.4	43.0	61.0	H.C.V.D. with uremia
W. G.	80	F	60.5	29.6	49.0	A.S.H.D.
C. R. (2)	38	F	54.5	29.8	54.6	H.C.V.D.
Mean	49.8		57.8	32.6	56.4	
S.D.					± 5.8	
B. With peripheral edema						
B. A.	45	F	46.8	26.5	56.6	Rheum. ht. disease
C. P.	71	M	59.6	33.6	56.4	A.S.H.D.
A. G.	62	M	73.4	44.0	60.0	H.C.V.D.
M. S.	74	F	78.5	27.6	35.2	H.C.V.D. (very obese; little edema)
D. M.	61	F	69.4	34.0	49.0	Cor. pulmonale
P. D.	47	M	70.0	41.7	59.5	Rh. ht. disease
H. M.	44	F	56.7	53.2	65.0	H.C.V.D.
S. W.	40	F	55.5	36.9	66.5	H.C.V.D.
Mean	55.5		63.7	37.1	56.0	
S.D.					±10.6	
C. With pulmonary congestion but no peripheral edema						
J. R.	59	F	82.0	34.4	42.0	A.S.H.D. Emphysema
F. C.	74	M	102.3	35.4	34.6	Myocardial infarct.
M. K.	34	F	51.7	35.2	68.0	H.C.V.D.
M. G.	53	F	61.4	27.5	44.8	H.C.V.D.
Mean	55		74.4	33.1	47.4	
S.D.					±14.3	

Pace, Kline, Schachman, and Harfenist (10)[3] and 0.27 is the fraction of solids in wet tissue. The carcass weight was taken as 56.5% after subtracting bone minerals. Inserting these values in the formula above, the total body water one would expect to observe with tritium is then approximately 74.5% of the lean body mass. The correction factor representing the maximum error, *i.e.*, for a lean subject, is 0.73/0.745 = 0.98. The difference of about 2% corresponds closely with the average of 2.3% in the differences in body water volumes observed when antipyrine is compared with tritium, as may be seen in Table II. This correction is not made in Tables II–V, because its exact value depends upon a more exact determination of lean tissue mass in individual cases.

Two facts are worthy of note. First, the magnitude of the error is not changed by the degree of obesity; and second, in edematous states, the error decreases as edema increases. The first is true because adipose tissue does not alter significantly either the amount of exchangeable hydrogen atoms or the volume of body water. The second is evident because as body water increases, the amount of tritium exchanged remains the same.

DISCUSSION

Radiation dosage is always an important consideration in the utilization of radioactive tracers. Two mc. of T_2O were selected for use in these

[3] The value used here is derived from Pace's figures for the nitrogen content of dry, fat-free tissue.

TABLE V

Total body water of patients with portal cirrhosis

Patient	Age	Sex	Weight (*Kg.*)	Body water (*liters*)	Body water (%)	
A. Without ascites or edema						
S. S.	70	F	52.0	19.4	37.3	
E. K.	33	F	45.6	21.5	47.2	
M. B.	61	F	53.2	21.1	39.7	
A. P.	30	F	81.8	30.9	50.0	
Mean	48.5		58.2	23.2	43.5	
S.D.					± 6.0	
B. With ascites and/or edema						
M. H.	36	F	71.9	37.8	52.6	Edema
A. B.	60	M	52.4	31.8	60.7	Ascites
M. T.	65	F	80.7	59.0	63.0	Ascites and anasarca
H. M.	51	M	49.6	26.6	53.7	Ascites
J. E.	47	M	71.3	40.2	56.4	Ascites and edema
J. E.	54	M	59.5	31.5	53.0	Ascites
W. D.	60	M	58.1	30.4	52.3	Ascites
Mean	53.3		63.3	36.8	56.0	
S.D.					± 4.3	

studies because they would produce a very low radiation dose; yet the specific activity of plasma (body water) gave a counting rate that was greater than background by a factor of 20 to 30.

Dosage rate and total dose were calculated for a 70 kilogram man using an average beta particle energy of 6 kilovolts and an exponential disappearance of tritium with a half life of 10 days.

The maximum dosage rate, which would occur at the time of injection, is: dosage rate = (2) (3.7×10^7) (6,000) $(1.64 \times 10^{-12})/(70) = 0.01$ rep./day; where 3.7×10^7 is the number of disintegrations per millicurie sec., and 1.64×10^{-12} is a factor for conversion from electron volts per second to roentgen equivalent physical (rep.) per day, assuming 84 ergs per rep.

Total dose was calculated by the formula:

$$\text{Total dose} = \text{dosage rate} \times \text{half time}/\ln 2 = (0.01)(10)/(0.693) = 0.14 \text{ rep.},$$

which represents the integration of the dosage rate from the time of injection to infinity. The radiation dose during the first week is considerably less than the generally accepted tolerance level of 0.3 roentgen per week.

The average total body water of the four young normal subjects, given in Table II, was 60.4% of body weight with tritium and 57.1% with antipyrine. The older subjects in the normal group, *i.e.*, between 34 and 56 years, in Table III, were found to have somewhat smaller volumes, averaging 52.1% of body weight, than is usually observed in the late 'teens and early twenties. This difference is undoubtedly greatly influenced by the difference in physical condition of the individuals measured in the two age groups, and is reflected in a correspondingly higher percentage of fat. There is at present, too little data to establish a reliable correlation between body water and age. It is felt that such a correlation would, in any event, be essentially a correlation between fat and age.

Since individuals differ markedly in degree of obesity, the proportion of body water normally present will vary accordingly and thus may mask any positive indication of altered water balance. Body water determinations in disease states consequently appear to be of little value as a quantitative measure of water retention or deficiency unless total fat can be determined independently. As Steele, Berger, Dunning, and Brodie (17) have already stated, an obese person can develop edema and still have a smaller proportion of body water than a lean, normal individual. This explains the lack of a significant difference in the proportion of body water observed in cardiac patients with and without edema, shown in Table IV.

The average percentage of total body water of Group A (Table V), which consists wholly of females, was 43.5% as compared to 44.6% for normal females (17). In Group B there are five male patients with an average body water of 55.2%, which is only slightly higher than our figure (52.1%) for normal males. The two females in this group had total body waters considerably above the average for normal females.

Little emphasis was placed on the measurement of body water turnover in this investigation because of previous work on this aspect of the problem by Pinson and Anderson (12). These investigators, using tritium, found biological half-lives of body water in eight normal subjects on an *ad libitum* water intake to range from nine to 14 days. These values agree closely with those found by Schloerb, and his associates (11) using deuterium. When the water intake of one of these individuals was

increased from 2.7 liters/day to 12.8 liters/day, the biological half-life was reduced from 10 days to 2.5 days, illustrating the lability of body water turnover in normal individuals.

The difficulties that confront efforts to interpret body water metabolism in disease states could doubtless be resolved if body fat could be determined at the same time. It seems reasonable that if body water can be expressed relative to lean body mass, its significance in disease could be more clearly defined. Although a method now in development for measuring body fat may be applicable to patients, the existing methods are either not sufficiently reliable, or they are impractical for use with sick subjects.

SUMMARY

1. Total body water has been determined with tritium labeled water in 57 individuals. Twenty were normal and the remainder were suffering from a variety of disease states.

2. Tritium appears to measure the same water volume as deuterium, averaging 2 to 4% higher than with antipyrine, and undergoes a similar degree of exchange with organic molecules as does deuterium.

3. The difference between antipyrine and tritium body water volumes is accounted for by exchange of tritium with protein bound hydrogen.

4. Body water determinations vary greatly from individual to individual, the variations in proportion reflecting for the most part variation in body fat. In pathologic states, where the lean body mass cannot be assumed to remain constant in composition, body water determinations are of limited value without a method for quantitating fat.

REFERENCES

1. Siri, W., Unpublished data.
2. Robinson, C. V., A methane, proportional counting method for the assay of tritium. Rev. Scient. Instruments, 1951, **22**, 353.
3. Mitchell, H. H., Hamilton, T. S., Steggerda, F. R., and Bean, H. W., The chemical composition of the adult human body and its bearing on the biochemistry of growth. J. Biol. Chem., 1945, **158**, 625.
4. Lavietes, P. H., D'Esopo, L. M., and Harrison, H. E., The water and base balance of the body. J. Clin. Invest., 1935, **14**, 251.
5. Rathbun, E., and Pace, N., Studies on body composition. I. Determination of total body fat by means of the body specific gravity. J. Biol. Chem., 1945, **158**, 667.
6. Osserman, E. F., Pitts, G. C., Welham, W. C., and Behnke, A. R., *In vivo* measurement of body fat and body water in a group of normal men. J. Appl. Physiol., 1950, **2**, 633.
7. Levitt, M. F., and Gaudino, M., Measurements of body water compartments. Am. J. Med., 1950, **9**, 208.
8. DaCosta, E., and Clayton, R., Report of the fat and water content and the specific gravity of the total carcass of the albino rat in relation to dietary restriction and rehabilitation. U. S. Army Medical Nutrition Laboratory, Chicago, Report No. 62, Sept., 1949.
9. Morales, M. F., Rathbun, E. N., Smith, R. E., and Pace, N., Studies on body composition. II. Theoretical considerations regarding major body tissue components with suggestions for application to man. J. Biol. Chem., 1945, **158**, 677.
10. Pace, N., Kline, L., Schachman, H. K., and Harfenist, M., Studies on body composition. IV. Use of radioactive hydrogen for measurement *in vivo* of total body water. J. Biol. Chem., 1947, **168**, 459.
11. Schloerb, P. R., Friis-Hansen, B. J., Edelman, I. S., Solomon, A. K., and Moore, F. D., The measurement of total body water in the human subject by deuterium oxide dilution. J. Clin. Invest., 1950, **29**, 1296.
12. Pinson, E. A., and Anderson, E. C., The body absorption, distribution and excretion of tritium in man and animals. The Atomic Energy Commission. Report LA-1218, March 12, 1951.
13. Brodie, B. B., Axelrod, J., Soberman, R., and Levy, B. B., The estimation of antipyrine in biological materials. J. Biol. Chem., 1949, **179**, 25.
14. Soberman, R., Brodie, B. B., Levy, B. B., Axelrod, J., Hallander, V., and Steele, J. M., The use of antipyrine in the measurement of total body water in man. J. Biol. Chem. 1949, **179**, 31.
15. Pace, N., Unpublished data.
16. Ussing, H. H., The exchange of H and D atoms between water and protein *in vivo* and *in vitro*. Skandinav. Arch. f. Physiol., 1938, **78**, 225.
17. Steele, J. M., Berger, E. Y., Dunning, M. F., and Brodie, B. B., Total body water in man. Am. J. Physiol., 1950, **162**, 313.

POSTOPERATIVE POTASSIUM DEFICIT AND METABOLIC ALKALOSIS. THE PATHOGENIC SIGNIFICANCE OF OPERATIVE TRAUMA AND OF POTASSIUM AND PHOSPHORUS DEPRIVATION [1]

By LEONARD P. ELIEL,[2, 3] OLOF H. PEARSON,[2] AND FREDERICK C. WHITE [4]

(From the Division of Clinical Investigation, Sloan-Kettering Institute, Memorial Center for Cancer and Allied Diseases, New York City)

(Submitted for publication December 6, 1951; accepted February 11, 1952)

Patients maintained postoperatively on parenteral fluids or a dietary intake low or lacking in potassium may develop a syndrome characterized by apathy, lethargy, muscular weakness, abdominal distension and ileus, cardiac arrhythmias and edema; and occasionally by confusion, delirium, muscular twitching and tetany. A clinical description of the syndrome as observed in 32 postoperative patients has appeared elsewhere (1). Evidence was found in these patients for the existence of potassium deficit, namely: *1*) the presence of hypopotassemia, hypochloremia and metabolic alkalosis, *2*) electrocardiographic changes consistent with potassium deficit, *3*) extensive expansion of the radiosodium space beyond the confines of the bromide space, suggesting that sodium had migrated into the cells to replace lost potassium, *4*) lowering of erythrocyte potassium and increase of erythrocyte sodium concentrations in several patients, and *5*) prompt reversal of the clinical and blood chemical abnormalities upon administration of adequate amounts of potassium.

Two factors were thought to be of primary importance in the pathogenesis of the potassium deficit in the group studied: *1*) adrenal cortical hyperfunction resulting from trauma ("alarm reaction") and leading to increased renal excretion of potassium and *2*) a low potassium intake with poor renal conservation of potassium. The first factor was suggested by the fact that muscular weakness, hypopotassemia, hypochloremia, and metabolic alkalosis are often observed in patients with Cushing's syndrome or in patients receiving ACTH (adrenocorticotropic hormone) or cortisone (1, 2); the second by the fact that all the postoperative patients but one were on a low potassium intake when the syndrome developed.

The two studies described in this paper were undertaken to evaluate the relative roles of low potassium intake and operative trauma in the production of the syndrome. Adrenal cortical hyperfunction, as estimated by several criteria, developed following the operative trauma in each case but was not separately evaluated in these studies. Hyperadrenocortical states have been induced in patients with neoplastic and other diseases by administration of adrenocorticotropic hormone (ACTH) or cortisone (11-dehydro-17-hydroxycorticosterone); and the resulting metabolic effects have been reported in detail (*2*–4).

The first study (case 1, N. M.) was designed to reproduce approximately the conditions under which the syndrome had been observed to develop, namely, simultaneous deprivation at the time of operation of potassium and phosphorus. These elements were later restored one at a time. In the second study (case 2, M. B.) a period of potassium and phosphorus deprivation was imposed preoperatively, while the operation was performed during a period when these elements were provided in adequate amounts. Both patients underwent major surgery for suspected or proved neoplasms.[5]

[1] This study was supported by grants from the National Cancer Institute of the Public Health Service C-925 (C2); jointly by the Office of Naval Research Contract no. N8-onr-66801, and the Atomic Energy Commission, and by a grant from the American Cancer Society.

[2] Damon Runyon Senior Clinical Research Fellow.

[3] Present address: Oklahoma Medical Research Institute, Oklahoma City 4, Oklahoma.

[4] Research Fellow in the Sloan-Kettering Institute.

[5] We are indebted to Dr. Gordon McNeer and the Gastric and Mixed Tumor Service of Memorial Center, for the opportunity to study these patients.

MATERIALS AND METHODS

Intravenous infusions were used exclusively except that tap water was permitted *ad libitum* by mouth. The parenteral fluids consisted of 1,000 cc. 10% amino acids solution,[6] 2,000 cc. 15% dextrose solution in distilled water, and 500 cc. 10% dextrose solution in physiologic saline, daily. To the 15% dextrose solution were added four ampules of buffered potassium phosphate solution,[7] and vitamins.[8] In case 1, 500 cc. of the amino acids solution were run in alternately with 1,000 cc. of 15% dextrose. In case 2, the solutions were infused simultaneously through a common needle, the flows being regulated so that all bottles would empty at approximately the same time.

Each bottle of solution was weighed to the nearest 0.5 gm., before and after infusion. Weights were converted to volume and the nitrogen, glucose, and electrolyte intakes calculated using analytical values found in our laboratory. The average time for administration of the fluids was 10 hours, during which the patient was kept in bed. The patients were otherwise ambulatory and took an approximately uniform amount of exercise before and after the infusions except on the operative day and subsequent two or three days when movement was limited by pain. They arose, however, on the first postoperative day for weighing and "dangling," and thereafter became ambulatory.

Feces from the preoperative and postoperative periods were pooled separately, carmine being used to mark the termination of each period. Feces were very small in volume and had to be obtained by distilled water enemas. Any changes in fecal excretion are probably, therefore, of doubtful significance. Stool chloride analyses were omitted in the second study. All vomitus and gastrointestinal fluids were collected and analyzed. Wound drainage was collected in a small bottle firmly taped to the draining orifice and likewise analyzed.

The total intake and output of calcium, phosphorus, nitrogen, potassium, sodium and chloride were determined by methods previously described (5). Gastrointestinal fluids and wound drainage were treated in the same manner as diets and feces. The methods used in the analyses of muscle tissue and erythrocytes; of serum sodium, potassium, magnesium, chloride, calcium, phosphorus, total protein, carbon dioxide content, and pH; of blood glucose; and of urinary creatine, creatinine, uric acid, glucose, and steroids[9] have been described elsewhere (1, 5), as have methods for performing dilution studies with radioactive sodium (Na^{24}) and bromide. The radiosodium and bromide spaces were determined at intervals of four and 5½ hours after the initial infusion. The sodium and chloride content of the spaces was calculated assuming that bromide ion has the same distribution as chloride (6). Blood urea nitrogen was determined by the method of Van Slyke and Plazin (7).

A muscle biopsy was taken from the rectus abdominis immediately after the initial operative incision in case 1. A repeat biopsy from the pectoral muscle was performed on the 11th postoperative day. Tissue water and electrolyte distributions (Table II) were computed assuming that chloride occupies an extracellular position. The possible inaccuracy of such an assumption is recognized, but it does provide a reasonable, if only approximate, description of water and electrolyte shifts.

All analyses were performed in duplicate.

Theoretical weight curves (Figures 3 and 7) were computed by the method of Reifenstein, Albright, and Wells (8). Intracellular balances of sodium, potassium, and phosphorus (Figures 9, 11 and Table I) were calculated in the manner described by Bartter and associates (9), except that in case 1 the ratios, K/N = 2.9 and P/N = .054, were derived from the patient's first muscle biopsy (Table II); and in case 2, the ratios, K/N = 2.8 and P/N = .064, were derived from the means of muscle analyses in five chronically ill hospital patients who had no evidence of electrolyte disturbance (4). In computing intracellular balances, corrections for insensible skin losses were made by subtracting algebraically the average of the control period balances from the found balance values.

DESCRIPTION OF CASES

Case 1 (N. M.) No. 95379. A 71 year old man was admitted to the metabolic ward three weeks after a cholecystojejunostomy for obstructive jaundice due to a mass in the head of the pancreas. Examination showed the patient to be well nourished. The jaundice had subsided. He was placed on complete parenteral feedings. After eight days on this regime a duodenectomy, partial pancreatectomy, partial gastrectomy, pancreatico-jejunostomy, choledochojejunostomy, and liver biopsy were performed under nitrous oxide-ether anesthesia. A 2 cm. mass was found in the region of the ampulla, which proved to be carcinoma on pathological examination. The duration of the operation was six hours. The patient was never in shock. Fifteen hundred cc. of citrated whole blood, estimated to replace operative blood losses, were administered intravenously during the operation.

Postoperatively, potassium phosphate was omitted from the parenteral feedings. Potassium chloride[10] was added on the ninth postoperative day and was replaced by potassium phosphate in equivalent amounts on the 12th day.

[6] Provided through the generosity of Dr. Augustus Gibson, Merck & Co., Rahway, New Jersey.

[7] Provided through the generosity of Abbott Laboratories, North Chicago, Illinois. Each ampule contained K_2HPO_4 (1.55 gm.) and KH_2PO_4 (0.30 gm.), providing 20 milliequivalents of potassium and 0.342 gm. of phosphorus.

[8] Thiamin 5 mg., niacin 50 mg., calcium pantothenate 5 mg., pyridoxine 5 mg., riboflavin 2 mg., vitamin B_{12} (30 gamma every three days), ascorbic acid 500 mg., and 2-methyl naphthoquinone 2.4 mg.

[9] Urinary ketosteroids, formaldehydogenic steroids and reducing steroids were determined in the laboratory of Dr. Konrad Dobriner.

[10] Potassium chloride solution in ampules was provided through the generosity of Abbott Laboratories.

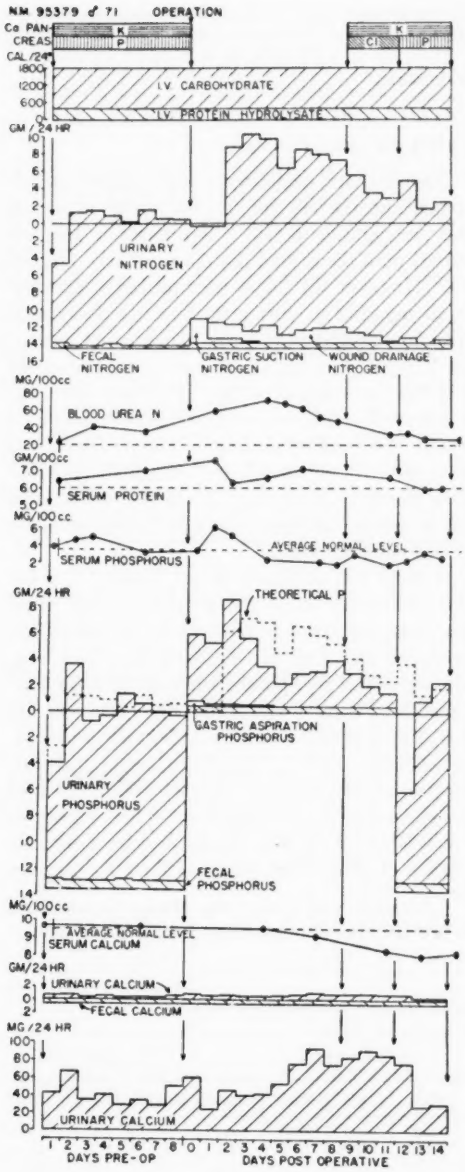


FIG. 1. CASE 1. THE EFFECTS OF OPERATIVE TRAUMA ON THE NITROGEN, PHOSPHORUS, AND CALCIUM BALANCES; ON THE SERUM TOTAL PROTEIN, PHOSPHORUS, AND CALCIUM CONCENTRATIONS; ON THE BLOOD UREA NITROGEN CONCENTRATION; AND ON THE URINARY CALCIUM EXCRETION

The patient remained in good condition throughout the postoperative period. A nasogastric tube was left in place two days. Except on the operative day, the gastrointestinal fluid losses were small. Wound drainage was remarkably scanty, no fistula developed, and wound healing proceeded satisfactorily. Peristalsis was present on the third day, but on the fourth day he felt very tired and was drowsy. On the eighth day some abdominal distension was noted. Improvement in the tiredness, drowsiness and abdominal distension occurred when potassium was provided. Rectal temperatures with daily maximums of 100.5° – 102° F. occurred postoperatively for which no cause was found and which persisted in spite of administration of penicillin and streptomycin. The fever subsided abruptly at the time antibiotics were discontinued on the 12th postoperative day.

The blood chemical findings during the balance study are charted in Figures 1 to 4. The serum calcium fell postoperatively, but the calculated serum ionized calcium concentration remained essentially constant due to a fall in serum protein concentration. The serum magnesium rose slightly postoperatively, then fell, but remained within the normal range. The blood urea nitrogen rose postoperatively, reaching 71.6 mg./100 cc. on the fourth postoperative day, then subsided gradually to control period values.

The electrocardiogram, which was normal preoperatively, showed slight lowering of T waves in all leads and depression of RT segments on the first postoperative day. On subsequent tracings (fourth to ninth days) these changes became more pronounced, the QT interval became prolonged and numerous premature ventricular contractions appeared. All of the abnormalities were corrected upon provision of potassium.

The packed red cell volume was 37% preoperatively, 44% immediately postoperatively and gradually fell to 28% on the day the study was terminated.

Case 2 (M. B.) No. 98585. A 55 year old woman developed, six weeks before entry, epigastric pain, nausea, vomiting and, on two occasions, hematemesis. A gastric

FIG. 1. (*Continued*)

The balance charts are constructed by plotting the intake downwards from the zero line and by plotting upwards from the intake, first the fecal, then the urinary excretions. If the sum of the excretions coincides with the zero line, balance is indicated; if it rises above, the balance is negative; and if it falls below, leaving a clear space, the balance is positive. The ordinates of the nitrogen, phosphorus, and calcium charts are so chosen that equal heights represent the approximate ratios at which these elements exist in normal protoplasm and bone; that is, 15 gm. of nitrogen to 1 gm. of phosphorus (protoplasm), and 2 gm. of calcium to 1 gm. of phosphorus (bone). The sum of the nitrogen and calcium balances gives the theoretical phosphorus balance (broken line); that is, the phosphorus balance that would be predicted from changes in protoplasm based on nitrogen, plus changes in bone based on calcium.

TABLE I

Intracellular balances of potassium, phosphorus, and sodium in case 1

Period	K	Cumulative intracell. K balance	P	Cumulative intracell. P balance	Na	Cumulative intracell. Na balance
	meq./period	meq.	meq./period	meq.	meq./period	meq.
1	+5		0		−21	
2	−9		−3		+29	
3	+4	0	+3	0	−6	+2
Op.						
1	−201	−201	−86	−86	+249	+251
2	−64	−265	+20	−66	+43	+294
3	−21	−286	+21	−45	−20	+274
4	+135	−151	+5	−40	−195	+79
5	+87	−64	+47	+7	+49	+128

ulcer was demonstrated radiographically and the patient was admitted for surgical exploration. Examination was normal except for some epigastric tenderness. She was placed on complete parenteral feedings. From the fifth to the eighth days, inclusive, potassium and phosphate ions were omitted. On the sixth day she complained of weakness and increased intestinal gas. Decreased peristalsis was noted on the eighth day. From the ninth day to the end of the study, potassium and phosphate ions were restored. On the tenth day the patient felt stronger and peristaltic sounds were more numerous. On the 13th day a sub-total gastrectomy was performed under nitrous oxide-ether anesthesia. A benign gastric ulcer was found. The operation lasted 4½ hours. The patient was not in shock at any time. She received 500 cc. of citrated whole blood during the operation. During the first two postoperative days there was moderate nausea, vomiting, abdominal distension, and a maximum rectal temperature of 100.2° F., but her postoperative course was uneventful thereafter.

The serum chemistries are charted in Figures 5 to 8. The serum calcium fell substantially after surgery, but the calculated ionized calcium remained essentially constant due to a fall in serum total protein concentration. No significant changes occurred in the electrocardiogram. No tissue analyses or dilution studies were done.

There was no significant change in the packed volume of erythrocytes.

EXPERIMENTAL RESULTS

Case 1 (N. M.)

Approximate equilibrium with respect to nitrogen and the electrolytes was observed during the preoperative period. No nitrogen loss occurred until the second postoperative day (Figure 1). However the blood urea nitrogen increased by 37 mg./100 cc. during the first five post-operative days (Figure 1), a rise which could account for 10.6 gm. of retained nitrogen.[11] On the second and third postoperative days, however, there was a markedly negative nitrogen balance, following which the urinary nitrogen losses diminished gradually. A coincident gradual fall in the blood urea nitrogen occurred. A slightly more rapid fall in urinary nitrogen was noted after provision of potassium chloride.

A large loss of phosphorus (Figure 1) in excess of that predicted from the calcium and nitrogen balances (broken line) occurred during the operative and first two postoperative days, suggesting loss of phosphorus from intracellular fluid (see also Figure 9 and Table I). This was followed by a period when more phosphorus was retained than would be predicted, suggesting that destruction of protoplasm was providing sufficient phosphorus to repair the deficit. When phosphate ion was added on the 12th postoperative day, retention of phosphorus occurred for only one day,

[11] Assuming body water to be 28.6 liters (53% body weight [10]) and urea to be distributed evenly therein.

TABLE II

Muscle and erythrocyte analyses at operation and 11 days postoperatively in case 1

Day	Tissue	Found values*					Derived data				Water distribution‡		
		$[K]_t$	$[Na]_t$	$[Cl]_t$	$[N]_t$	$[P]_t$	$[K]_i$	$(K)_i$	$[Na]_i$	$(Na)_i$	$(H_2O)_t$	$(H_2O)_e$	$(H_2O)_i$
		meq.	meq.	meq.	gm.	gm.	meq.*	meq.†	meq.*	meq.†	gm.	gm.	gm.
0	Muscle	437.3	163.6	98.3	134.4	7.24	433.8	148.8	43.0	14.7	791	177	614
11	Muscle	258.8	161.3	116.5	141.3	7.69	255.2	122.4	10.3	4.9	759	249	510
0	Erythrocytes	108.6	9.0	81.6									
11	Erythrocytes	86.0	—	73.6									

Subscripts t, i, e, signify total tissue, intracellular and extracellular, respectively.
* Per kilogram dry, fat-free muscle or per liter packed erythrocytes.
† Per kilogram of intracellular water.
‡ Per kilogram wet, fat-free tissue.

suggesting that the intracellular deficit had been largely repaired. This interpretation is confirmed by the analysis of muscle tissue, taken one day before phosphorus was started, which shows essentially no change in the phosphorus content (Table II). The extracellular phosphorus concentration fell to abnormally low levels, a fall which was more than could be accounted for by the apparent expansion in extracellular fluid which occurred simultaneously (see below). The hypophosphatemia was corrected following provision of phosphate ion.

The urinary calcium excretion (Figure 1) rose during the period of phosphate deprivation, falling to control period levels only on the second day of phosphate readministration. Thus, as a result of increased bone destruction, a small amount of phosphorus (equivalent to the increase in negative calcium balance in Figure 1) was made available for reduction and repair of intracellular phosphorus losses. When the theoretical phosphorus balance finally coincided with the actual, indicating tissue repletion, the urinary calcium excretion fell to its control period values.

The potassium balance (Figure 2), like the phosphorus balance, shows postoperatively, large losses in excess of those predicted from the nitrogen balance (broken line), suggesting intracellular depletion. This is also shown by the calculated intracellular balances (Table I and Figure 9) and is confirmed by the postoperative muscle analysis (Table II) which shows a drop of 178.6 meq./kg. of dry, fat-free tissue. From such a marked drop one would have anticipated an intracellular deficit of around 700 meq.[12] instead of the 151 to 286 meq. predicted from the balance data (Table I). Two explanations for the discrepancy are suggested: *1*) The patient's muscles were found to be extraordinarily thin at both biopsies and large areas had to be excised to obtain sufficient tissue. A low muscle mass was also suggested by the creatinine excretion which averaged only 17.8 mg./kg./day during the control period. The smaller the muscle mass the larger would be the fall in its potassium content, per unit weight, produced by a given potassium loss. *2*) A transfer of potassium from muscle to liver glycogen may have occurred, as

[12] Calculated using muscle analyses (Table II), and assuming that the muscle mass is 41% of body weight (11), and that other tissues were not significantly depleted.

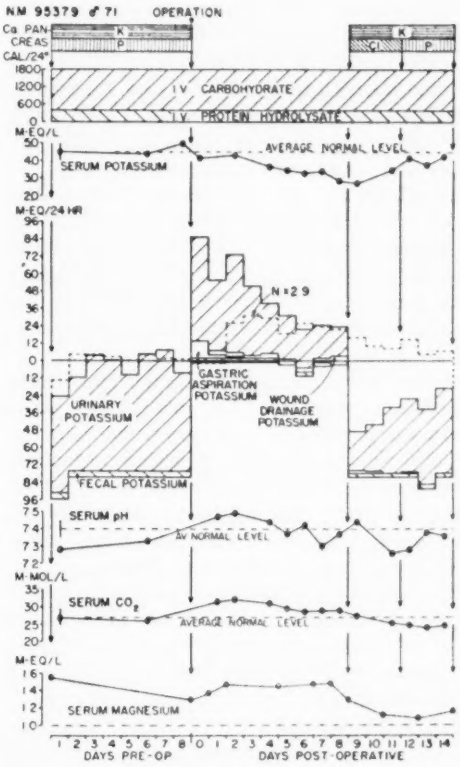


FIG. 2. CASE 1. THE EFFECTS OF OPERATIVE TRAUMA AND POTASSIUM AND CHLORIDE DEPRIVATION ON THE POTASSIUM BALANCE; ON THE SERUM POTASSIUM AND MAGNESIUM CONCENTRATIONS; AND ON THE SERUM CARBON DIOXIDE CONTENT AND pH

suggested by Bartter and associates from observations on patients receiving adrenocorticotropic hormone (9).

The tissue analyses (Table II) demonstrated, in addition to a marked potassium depletion postoperatively, a shift of water from the intracellular to the extracellular compartment which in effect would tend to preserve the concentration of intracellular potassium. The failure to find increased tissue sodium associated with potassium deficit (Table II) may be explained by the fact that at the time the biopsy was taken (11th day, postoperative period 4), 73% of the previously accumulated intracellular sodium had been extruded.

The sodium space determined on the eighth postoperative day, period 3 (Figure 10), showed a marked extension over preoperative values. Its sodium content exceeded by 270 meq. the amount which could be accounted for in the bromide space (broken line), suggesting either intracellular migration of sodium or increased exchangeability of intracellular sodium. The former interpretation appears more likely, since the loss of intracellular potassium (286 meq.) and gain of intracellular sodium (274 meq.) calculated from the balance data at this time (Table I) fit with admirable precision the increase in intracellular sodium suggested by the dilution studies.

Unlike the phosphorus deficit, no repair of the intracellular potassium deficit occurred until potassium was provided. A pronounced fall in extracellular fluid potassium concentration occurred postoperatively, which could not be accounted for by extracellular fluid expansion. This was accompanied by the development of metabolic alkalosis which, however, subsided spontaneously. Addition of potassium resulted in prompt correction of the hypopotassemia and a further fall in the serum pH and carbon dioxide content. Dilution studies on the 15th postoperative day (Figure 10) also demonstrated a marked reduction of intracellular sodium (area above broken line), suggesting extrusion of sodium when potassium was provided.

The chloride balance (Figure 3) shows a marked rise in urinary chloride excretion on the operative day. Losses from gastric aspiration contributed to a large negative balance. The sodium balance on the operative day, although negative, shows a retention relative to chloride (broken line). The balance data indicate that the chloride loss was made up by the fifth postoperative day and that further retention occurred thereafter. The latter is confirmed by the dilution studies (Figure 10), which demonstrated on the eighth postoperative day an increase in the chloride content of the bromide space of about 300 meq. These data suggest that the metabolic alkalosis was at least partially corrected by repair of the chloride deficit and that further retention of sodium and chloride then occurred, resulting in expansion of extracellular fluid volume. The expansion of the sodium and chloride spaces also indicates that the increased retention of sodium and chloride shown by the balance data was not an apparent one due to insensible skin losses.

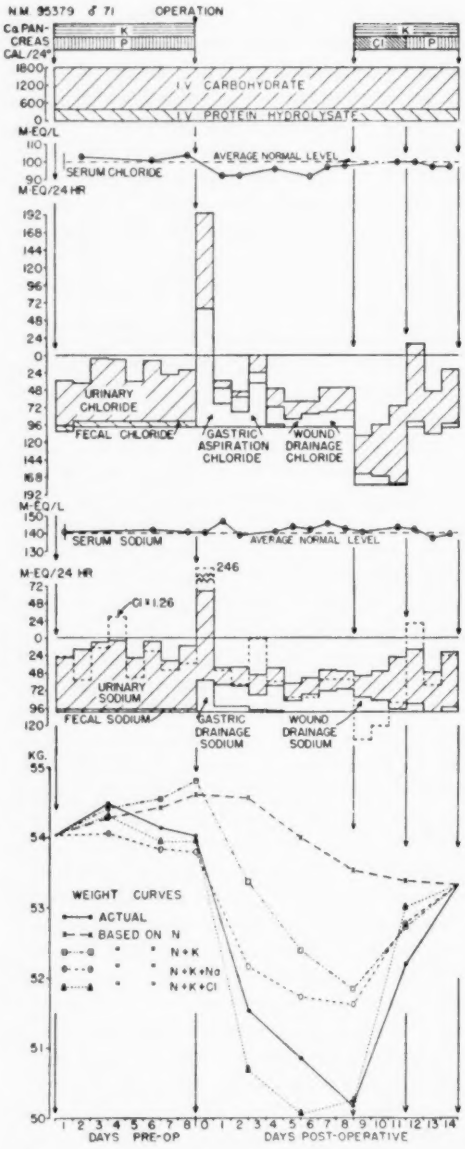


FIG. 3. CASE 1. THE EFFECTS OF OPERATIVE TRAUMA AND POTASSIUM AND CHLORIDE DEPRIVATION ON THE CHLORIDE AND SODIUM BALANCES; ON THE SERUM CHLORIDE AND SODIUM CONCENTRATIONS; AND ON THE ACTUAL AND THEORETICAL WEIGHT CURVES

The broken line on the sodium balance (Cl × 1.26) represents the sodium balance that would be expected if sodium were excreted in the same ratio to chloride as exists normally in extracellular water.

The theoretical weight curves (Figure 3) demonstrate that the actual weight changes are most closely approximated by a curve combining estimated changes in protoplasm, intracellular and extracellular fluids as calculated, respectively, from the nitrogen, potassium, and chloride balances (N + K + Cl). The curve which utilizes sodium instead of chloride (N + K + Na) predicts a smaller weight loss postoperatively, thus demonstrating again the retention of sodium relative to chloride which occurred at that time.

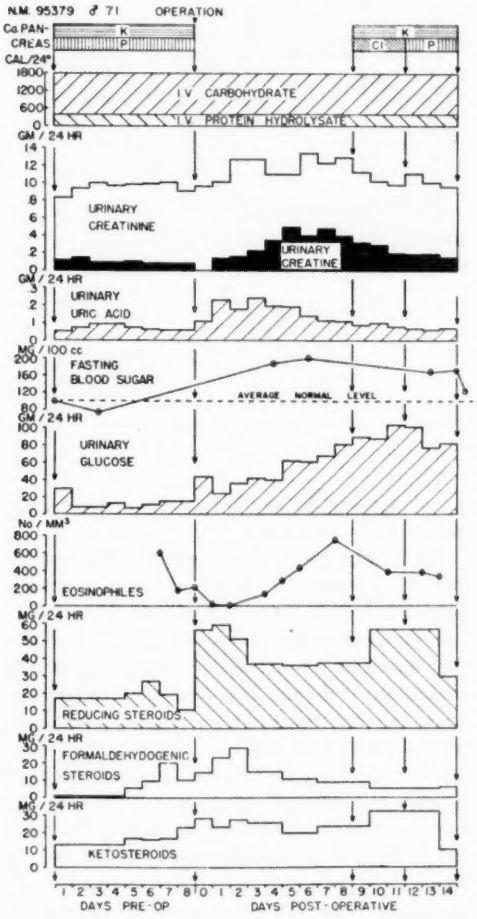


FIG. 4. CASE 1. THE EFFECTS OF OPERATIVE TRAUMA AND POTASSIUM AND PHOSPHORUS DEPRIVATION ON THE URINARY CREATINE, CREATININE, URIC ACID, GLUCOSE, REDUCING STEROID, FORMALDEHYDOGENIC STEROID, AND KETOSTEROID EXCRETION; AND ON THE FASTING BLOOD GLUCOSE AND EOSINOPHILE CONCENTRATIONS

The theoretical weight curves (Figure 3) demonstrate that the actual weight changes are most closely approximated by a curve combining estimated changes in protoplasm, intracellular and extracellular fluids as calculated, respectively, from the nitrogen, potassium, and chloride balances (N + K + Cl). The curve which utilizes sodium instead of chloride (N + K + Na) predicts a smaller weight loss postoperatively, thus demonstrating again the retention of sodium relative to chloride which occurred at that time.

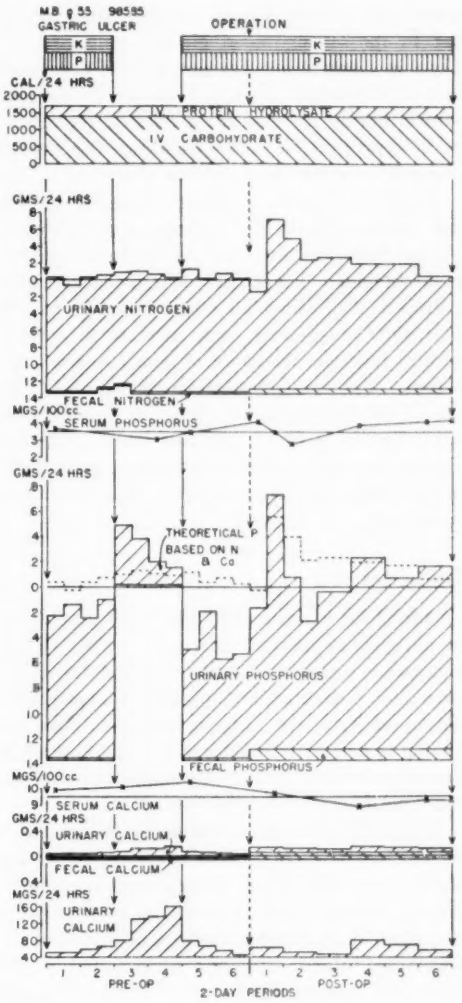


FIG. 5. CASE 2. THE EFFECTS OF POTASSIUM AND PHOSPHORUS DEPRIVATION AND OF OPERATIVE TRAUMA ON THE NITROGEN, PHOSPHORUS, AND CALCIUM BALANCES; ON THE URINARY CALCIUM EXCRETION; AND ON THE SERUM PHOSPHORUS AND CALCIUM CONCENTRATIONS

The urinary excretion of creatine and uric acid rose postoperatively (Figure 4). A progressive fall in urinary creatine occurred at the time that potassium chloride was restored to the intake. A progressive rise in urinary glucose occurred post-

operatively (Figure 4) and was associated with a rise in the fasting blood glucose concentration. There is a suggestion that provision of phosphate may have resulted in slight reduction of the glycosuria during the last three days of the study. Both hyperglycemia and glycosuria disappeared promptly on cessation of parenteral feeding.

A slight increase in the urinary reducing steroids and ketosteroids occurred preoperatively (Figure 4), but there was a marked rise in formaldehydogenic steroids. These rises were associated with a drop in the eosinophile count from 606 to 189 cells/mm.^{3}, suggesting some increase in adrenal cortical function, the cause of which was not apparent. The circulating eosinophile count (Figure 4) fell to zero on the first two postoperative days, then rose to preoperative levels or above. Further increases in steroid excretion, particularly in the reducing steroids, were observed postoperatively.

Case 2 (M. B.)

During the initial control period the patient was in nitrogen equilibrium (Figure 5). No significant rise in nitrogen excretion occurred during the period of potassium and phosphate deprivation. Postoperatively, the nitrogen excretion rose, resulting in a negative balance of 7.2 gm. on the first postoperative day, and remained elevated until the tenth postoperative day (period 5).

When phosphate was omitted preoperatively, a prompt negative phosphorus balance resulted and there was a slight fall in serum phosphorus concentration. Intracellular depletion of phosphorus is indicated by *a*) phosphorus loss in excess of the theoretical balance (Figure 5, broken line), *b*) the calculated intracellular balance (Figure 11), and *c*) retention of phosphorus in excess of the theoretical when the phosphorus intake was restored (Figure 5).

During the period of phosphorus deprivation, as in case 1, there was a marked increase in urinary calcium excretion which subsided when phosphorus was restored.

An abrupt rise in urinary phosphorus excretion occurred on the operative day, resulting in a negative balance on the first postoperative day, in slight excess of the theoretical balance. This was followed by a period of retention. A slightly negative phosphorus balance was observed during the last six days of the study.

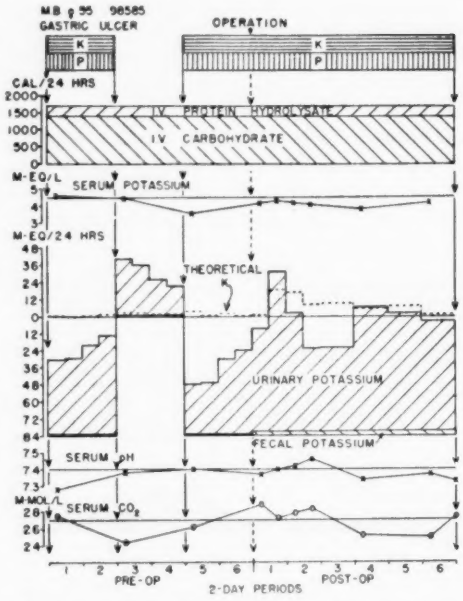


FIG. 6. CASE 2. THE EFFECTS OF POTASSIUM AND PHOSPHORUS DEPRIVATION AND OF OPERATIVE TRAUMA ON THE POTASSIUM BALANCE, SERUM POTASSIUM CONCENTRATION, SERUM CARBON DIOXIDE CONTENT, AND SERUM pH

The potassium balance (Figure 6) closely paralleled the phosphorus balance. During potassium deprivation, there was a loss of potassium of 40.8 meq. on the first day which diminished gradually to 22.0 meq. on the last day. This loss was largely intracellular (Figure 11). A simultaneous decrease in serum potassium concentration occurred, but there was no significant change in the serum pH or carbon dioxide content. Marked increases in urinary potassium excretion occurred on the first two postoperative days, exceeding the theoretical balance on the first postoperative day by 15 meq. A period of potassium retention ensued, suggesting repair of a deficit. A slight decrease in serum potassium concentration and a gradual rise in serum pH occurred postoperatively. The carbon dioxide content did not change significantly.

Retention of sodium and chloride occurred during potassium and phosphate deprivation (Figure

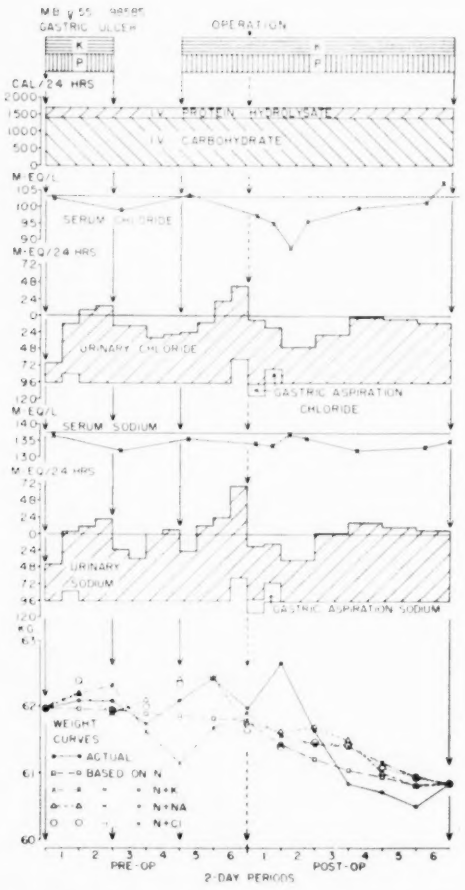


FIG. 7. CASE 2. THE EFFECTS OF POTASSIUM AND PHOSPHORUS DEPRIVATION AND OF OPERATIVE TRAUMA ON THE CHLORIDE AND SODIUM BALANCES, ON THE SERUM CHLORIDE AND SODIUM CONCENTRATIONS; AND ON THE ACTUAL AND THEORETICAL WEIGHT CURVES

7). Addition of potassium phosphate resulted in a moderate diuresis of sodium and chloride. Retention of sodium and chloride was again observed postoperatively. The intracellular balance (Figure 11) demonstrates the opposite movements across the cell membrane of sodium with respect to potassium during the period of potassium deprivation, and postoperatively.

The theoretical weight curves (Figure 7) reflect the intracellular potassium depletion and sodium and chloride retention during preoperative periods 3 and 4.

Moderate rises in urinary creatine and uric acid occurred postoperatively (Figure 8). Urinary glucose excretion (Figure 8) was elevated at the onset of the study, reached a maximum during the period of potassium and phosphate deprivation, then subsided to essentially normal amounts, with only a slight rise occurring postoperatively. The blood glucose level (Figure 8) fluctuated within normal limits. Circulating eosinophiles fell to zero on the first two postoperative days coin-

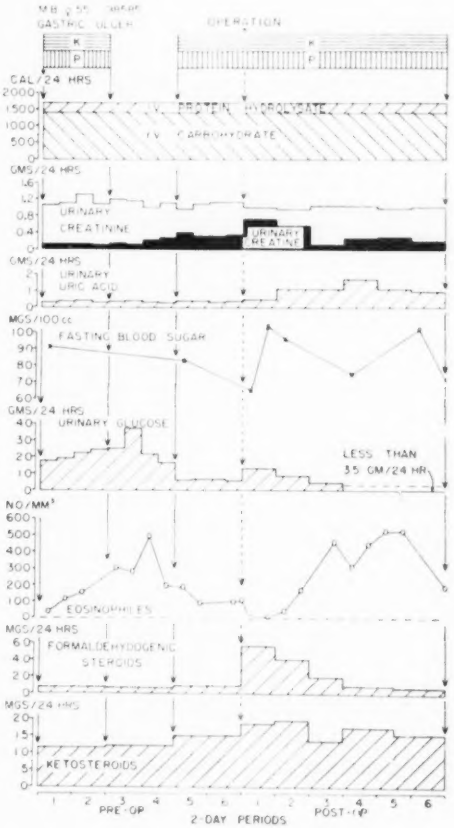


FIG. 8. CASE 2. THE EFFECTS OF POTASSIUM AND PHOSPHORUS DEPRIVATION AND OPERATIVE TRAUMA ON THE URINARY CREATINE, CREATININE, URIC ACID, GLUCOSE, FORMALDEHYDOGENIC STEROID, AND KETOSTEROID EXCRETION; AND ON THE FASTING BLOOD GLUCOSE AND EOSINOPHILE CONCENTRATIONS

cident with a marked rise in formaldehydogenic steroids and a slight elevation of the ketosteroids (Figure 8).

DISCUSSION

The postoperative eosinopenia and rise in urinary steroid excretion which occurred in these patients can be interpreted as evidence of increased adrenal cortical function. We have previously pointed out that the muscular weakness and hypopotassemia, hypochloremia, and metabolic alkalosis exhibited by certain postoperative patients bear a striking resemblance to the findings in patients with Cushing's syndrome (spontaneous hyperadrenocorticism) (12). Similarly, in patients in whom hyperadrenocorticism was induced by administration of ACTH or cortisone, we have observed, just as in cases 1 and 2, losses of nitrogen; intracellular depletion of potassium and phosphorus; the development of metabolic alkalosis, hypopotassemia and hypochloremia; rises in urinary creatine, uric acid, and steroids; and eosinopenia (3). Significant tissue deficits of potassium and phosphorus were also demonstrated both in a patient with Cushing's syndrome and in patients who had received ACTH or cortisone (4). It appears, therefore, that the postoperative metabolic changes could be accounted for by increased adrenal cortical function.

On the other hand, provision of potassium ion in case 1 was accompanied by diminished losses of nitrogen, phosphorus, potassium and creatine, suggesting that potassium deprivation may have played an important role in these losses. There is evidence that administration of potassium will reduce nitrogen loss in rats (13) or humans (14) receiving ACTH, or in patients with Cushing's syndrome (5).

When potassium and phosphorus were omitted, however, in case 2 prior to surgery, there was no significant change in the nitrogen balance. In a study reported elsewhere (3) in which a patient was maintained on an artificial potassium-free diet for 18 days, the maximum daily potassium loss was 29.9 meq. and no negative nitrogen balance resulted. The results indicate that short periods of potassium deprivation in the adult human do not cause nitrogen loss.

Potassium deprivation in case 2 produced a maximum daily loss of 38.1 meq. of potassium in excess of that expected from the nitrogen balance. A maximum daily postoperative loss of 85.7 meq. was observed, however, in case 1. The largest daily loss of potassium in excess of nitrogen, reported by several observers, in subjects on potassium-free intakes was 55 meq. (3, 15–17). Potassium losses following traumatic procedures vary widely but sometimes exceed this figure by substantial amounts, with losses up to 110 meq. in excess of nitrogen having been reported (18). It is evident that operative trauma in case 2 resulted in a marked increase in urinary potassium and a small intracellular potassium loss in spite of a constant potassium intake.

During the period of potassium phosphate deprivation in case 2 there was no significant increase in urinary creatine, uric acid or steroid excretion, nor any fall in eosinophile concentration. It seems unlikely, therefore, that the lack of these two ions

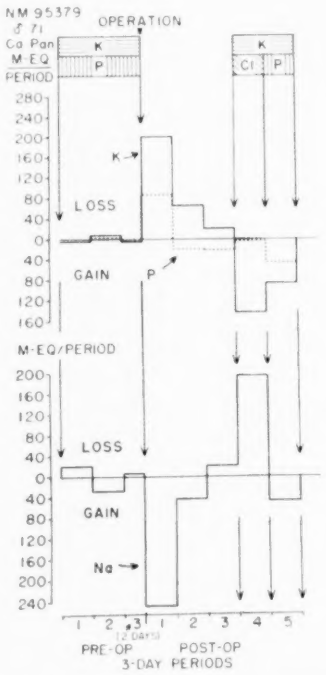


FIG. 9. CASE 1. THE EFFECTS OF OPERATIVE TRAUMA AND POTASSIUM AND PHOSPHORUS DEPRIVATION ON THE INTRACELLULAR BALANCES OF POTASSIUM, PHOSPHORUS, AND SODIUM

could entirely account for the metabolic changes observed postoperatively.

Following the operation in case 2, most of the metabolic changes seen in case 1 were duplicated, though they were in general of shorter duration and lesser magnitude. It is thus apparent that even though the potassium and phosphorus intakes were maintained, trauma resulted in the production of changes similar to those seen in hyperadrenocorticism.

The suggested sequence of events is that as a result of trauma, the pituitary produces more ACTH which in turn causes increased production of adrenal cortical steroids. The latter, either through effects on tissues, the kidney, or both, causes the described metabolic changes.

Some evidence indicates, however, that adrenal cortical hyperfunction is not necessarily associated with the post-traumatic "catabolic response." Stressed, adrenalectomized rats maintained with cortin or cortisone still exhibit a "catabolic response" as measured by non-protein nitrogen excretion (19) or blood urea nitrogen production (20). It has not been determined in the human, however, whether the metabolic changes, noted in the postoperative patients, would occur in the absence of adrenal cortical hyperfunction.

Werner, Habif, Randall, and Lockwood (21) have proposed that the nitrogen loss which occurs postoperatively is due to the reduction in caloric intake usually incident to an operative procedure. In the experiments reported here a constant caloric and protein intake was maintained pre- and postoperatively. This intake may, however, have become inadequate to meet the requirements of the injured or stressed organism. The moderate postoperative temperature elevation encountered in case 1 may also have contributed to a caloric deficit by increasing caloric expenditure. No significant postoperative temperature eleva-

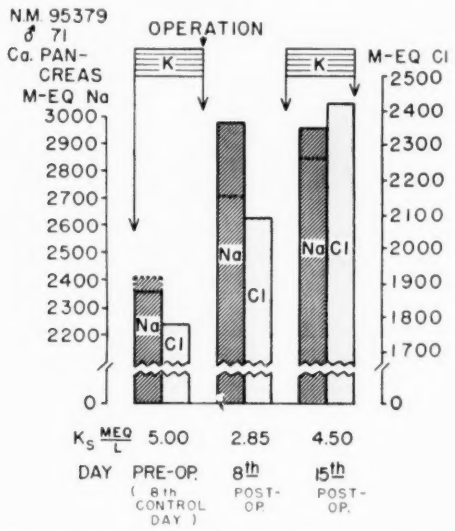


FIG. 10. CASE 1. THE EFFECTS OF OPERATIVE TRAUMA AND POTASSIUM AND PHOSPHORUS DEPRIVATION ON THE SODIUM AND CHLORIDE CONTENTS OF THE SODIUM AND BROMIDE SPACES, RESPECTIVELY

The chart is so constructed that equal heights of the paired columns represent the existence of sodium and chloride ions at their approximately normal concentration ratio of 1.26:1 in extracellular water. The broken lines represent the amount of sodium that could be accommodated in the simultaneously found bromide space at the existing concentration of sodium in extracellular water. Corresponding serum potassium concentrations are given below each determination.

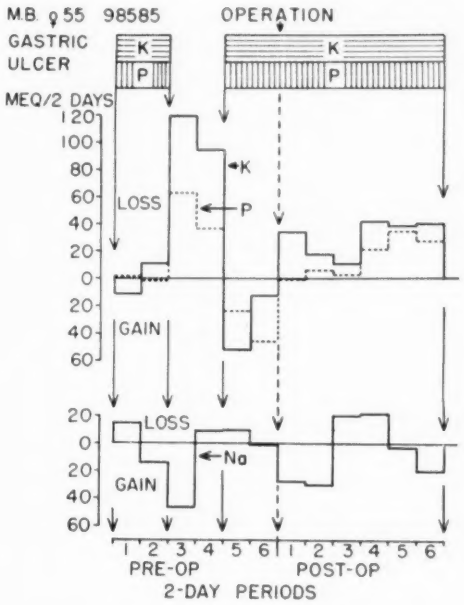


FIG. 11. CASE 2. THE EFFECTS OF POTASSIUM AND PHOSPHORUS DEPRIVATION AND OF OPERATIVE TRAUMA ON THE INTRACELLULAR BALANCES OF POTASSIUM, PHOSPHORUS, AND SODIUM

tion occurred in case 2, however, in whom there was nevertheless a definite "catabolic response." Doubling the dietary intake of a patient receiving ACTH will abolish markedly negative balances of nitrogen, phosphorus, and potassium and indeed cause retention of these elements (3). The protein catabolic response to stress in the rat can also be abolished by the administration of glucose or amino acids (22), or reduced by the administration of potassium (13). These observations suggest the possibility of abolishing post-traumatic metabolic changes if high enough intakes of calories, protein, and intracellular electrolytes could be achieved.

The high intravenous glucose intake was equivalent to a daily glucose tolerance test; normal preoperatively in case 1, but demonstrating, postoperatively, gradually increasing glycosuria and hyperglycemia. These manifestations, however, disappeared promptly when the glucose intake was reduced to normal at the termination of parenteral feeding. It seems unlikely that removal of the head of the pancreas could entirely account for these events; or that adrenal cortical hyperfunction could be implicated, since activity of the adrenal cortex appeared to be subsiding while the diabetic state was becoming worse. The increasing glycosuria correlates directly, however, with the cumulative intracellular potassium deficit (Table I). A relationship between potassium and carbohydrate metabolism has been demonstrated in a number of experimental studies. Bennett, Whitney, and Glafkides (13) have shown that potassium, given to partially depancreatized rats receiving ACTH, will reduce the glycosuria; and Gardner and associates (23) have demonstrated diabetic glucose tolerance curves in rats subjected to prolonged potassium deficit. Hastings, Teng, Nesbett, and Sinex have demonstrated that liver slices incubated in a medium containing a high concentration of potassium (110 meq./L.) show less glucose formation from pyruvate and more glucose uptake than do slices incubated in a medium with a low concentration of potassium (5 meq./L.). Glycogen formation from both glucose and pyruvate is higher in the high potassium medium than in the low potassium medium (24). It has furthermore been shown that potassium is necessary for the formation of phosphopyruvate, an intermediary in the carbohydrate cycle, and source of high energy phosphate bonds (25).

Thus it seems reasonable to suggest that potassium deficit could have resulted in impaired glucose utilization, and increased glycogenolysis and gluconeogenesis. No significant reduction in glycosuria occurred when potassium was added, but the marked retention of potassium in excess of that expected from the nitrogen balance during this period indicates that insufficient potassium had been retained, even at the end of the study, to repair the deficit (see also Table I).

Slight reduction in glycosuria coincided with addition of phosphate ion. One is inclined to speculate whether the phosphorus deficit resulted in impaired phosphorylating mechanisms and decreased glucose utilization. Two facts against this view are: *1*) the balance data which indicate that repair of the intracellular phosphorus deficit had already occurred at the peak of the diabetes and *2*) the tissue analysis, taken before phosphate was restored, which showed no decrease in phosphorus content.

Deprivation of phosphate ion in each case resulted in stepwise increases of urinary calcium excretion until the intracellular phosphate deficit was repaired. Provision of phosphate ion in each instance resulted in a prompt drop of urinary calcium. Increased calcium excretion due to immobilization seems unlikely, since moderate and uniform activity was maintained throughout each study, except for the immediate postoperative period.

SUMMARY AND CONCLUSIONS

1. The metabolic effects resulting from operative trauma and from potassium and phosphorus deprivation have been observed in two patients. During the postoperative period one of the patients received no potassium or phosphorus, while the other received optimal amounts of these elements. The patients were maintained on constant caloric and protein intakes.

2. Similar metabolic changes were observed in each patient postoperatively, though more pronounced in the patient who received no potassium or phosphorus. These changes were: *a*) loss of protoplasm as shown by negative balances of nitrogen, phosphorus, and potassium, *b*) intracellular depletion of potassium and phosphorus, *c*)

development of metabolic alkalosis and hypochloremia, *d*) eosinopenia, and *e*) increased urinary excretion of uric acid, creatine, formaldehydogenic steroids and ketosteroids.

3. These changes bear a striking resemblance to those seen in patients with hyperadrenocorticism, whether spontaneous or induced by ACTH or cortisone.

4. A preoperative period of potassium and phosphorus deprivation in one of the patients resulted in intracellular losses of these elements but failed to produce increased nitrogen loss or any evidence of increased adrenal cortical function.

5. In spite of substantial and constant intakes of potassium and phosphorus, operative trauma in case 2 resulted in marked increases in urinary content, and in intracellular losses, of these elements.

6. These findings suggest that a post-traumatic increase in adrenal cortical steroid production resulted in the metabolic changes observed. However, it has not been established whether similar changes with respect to nitrogen and the electrolytes would occur post-traumatically in the absence of a hyperadrenocortical state.

7. A possible relationship between postoperative potassium deficit and the development of a diabetic state in one of the patients is discussed.

8. A prompt rise in urinary calcium excretion was observed during phosphorus deprivation, indicating that in the presence of intracellular phosphorus deficit, decalcification of bone provides a small amount of phosphorus for repair purposes.

ACKNOWLEDGMENTS

The authors gratefully acknowledge helpful criticism of the manuscript by Dr. Henry T. Randall and the assistance of Milton Grossman, research fellow; Sophie Rys, Rhea Neznek, Albina Foster, Olive Pike, nurses; Edna Block, Ann Spilka, research assistants; Bernice Katz, Constance Ryder, Marguerite Oliver, technicians; and Bess Lande, cartographer.

REFERENCES

1. Eliel, L. P., Pearson, O. H., and Rawson, R. W., Postoperative potassium deficit and metabolic alkalosis. New England J. Med., 1950, **243**, 471 and 518.
2. Sprague, R. G., Power, M. H., Mason, H. L., Albert, A., Mathieson, D. R., Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F., Observations on the physiologic effects of cortisone and ACTH in man. Arch. Int. Med., 1950, **85**, 199.
3. Pearson, O. H., and Eliel, L. P., Experimental studies with ACTH and cortisone in patients with neoplastic disease; *in* Recent Progress in Hormone Research, edited by Pincus, G. Academic Press, New York, 1951, Vol. VI, p. 373.
4. Eliel, L. P., Hellman, L., Pearson, O. H., and Katz, B., The effects of ACTH on the electrolyte content of various body tissues; *in* Proceedings of the Second Clinical ACTH Conference, Vol. I, Research; edited by Mote, J. R. Blakiston Co., Philadelphia, 1951, p. 196.
5. Eliel, L. P., and Pearson, O. H., The metabolic effects of adrenocorticotropic hormone (ACTH) in a patient with Cushing's syndrome and acromegaly. J. Clin. Endocrinol., 1951, **11**, 913.
6. Brodie, B. B., and Friedman, M. M., Determination of bromide in tissues and biological fluids. J. Biol. Chem., 1938, **124**, 511.
7. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Vol. II, Methods. Williams & Wilkins Co., Baltimore, 1932, p. 558.
8. Reifenstein, E. C., Jr., Albright, F., and Wells, S. L., The accumulation, interpretation, and presentation of data pertaining to metabolic balances, notably those of calcium, phosphorus, and nitrogen. J. Clin. Endocrinol., 1945, **5**, 367.
9. Bartter, F. C., Fourman, P., Albright, F., Forbes, A. P., Jefferies, W. McK., Griswold, G., Dempsey, E., Bryant, D., and Carroll, E., The effect of adrenocorticotropic hormone in panhypopituitarism. J. Clin. Invest., 1950, **29**, 950.
10. Steele, J. M., Berger, E. Y., Dunning, M. F., and Brodie, B. B., Total body water in man. Am. J. Physiol., 1950, **162**, 313.
11. Shohl, A. T., Mineral Metabolism. Am. Chem. Soc. Monograph Series, Reinhold Publishing Corp., New York, 1939.
12. Pearson, O. H., and Eliel, L. P., Postoperative alkalosis and potassium deficiency. J. Clin. Invest., 1949, **28**, 803.
13. Bennett, L. L., Whitney, J. E., and Glaikides, C., Modification of the metabolic effects of adrenocorticotropic hormone and growth hormone by the administration of a diet high in potassium chloride. J. Clin. Endocrinol., 1950, **10**, 826.
14. Pearson, O. H., Eliel, L. P., and Rawson, R. W., Regression of lymphoid tumors in man induced by ACTH and cortisone; *in* Proceedings of the First Clinical ACTH Conference; edited by Mote, J. R. Blakiston Co., Philadelphia, 1950, p. 318.
15. Gamble, J. L., Physiological information gained from studies on the life raft ration. Harvey Lect., 1947, **42**, 247.
16. Tarail, R., and Elkinton, J. R., Potassium deficiency and the role of the kidney in its production. J. Clin. Invest., 1949, **28**, 99.

17. Randall, H. T., Habif, D. V., Lockwood, J. S., and Werner, S. C., Potassium deficiency in surgical patients. Surgery, 1949, **26**, 341.

18. Howard, J. E., and Bigham, R. S., Jr., Movement of potassium during the protein catabolic reaction after trauma. Tr. of Tenth Conf. on Metabolic Aspects of Convalescence. Josiah Macy, Jr. Foundation, New York, 1945, pp. 272–302.

19. Ingle, D. J., Some studies on the role of the adrenal cortex in organic metabolism. Ann. New York Acad. Sc., 1949, **50**, 576.

20. Engel, F. L., Comparative effects of ACTH and stress in nitrogen metabolism; *in* Proceedings of the Second Clinical ACTH Conference, Vol. I, Research; edited by Mote, J. R. Blakiston Co., Philadelphia, 1951, p. 235.

21. Werner, S. C., Habif, D. V., Randall, H. T., and Lockwood, J. S., Postoperative nitrogen loss: A comparison of the effects of trauma and of caloric readjustment. Ann. Surg., 1949, **130**, 688.

22. Engel, F. L., A consideration of the roles of the adrenal cortex and stress in the regulation of protein metabolism; *in* Recent Progress in Hormone Research, edited by Pincus, G. Academic Press, New York, 1951, Vol. VI., p. 277.

23. Gardner, L. I., Talbot, N. B., Cook, C. D., Berman, H., and Concepcion, U. R., The effect of potassium deficiency on carbohydrate metabolism. J. Lab. & Clin. Med., 1950, **35**, 592.

24. Hastings, A. B., Teng, C. T., Nesbett, F. B., and Sinex, F. M., Studies on carbohydrate metabolism in rat liver slices. I. The effect of cations in the media. J. Biol. Chem., 1952, **194**, 69.

25. Lardy, H. A., and Ziegler, J. A., The enzymatic synthesis of phosphopyruvate from pyruvate. J. Biol. Chem., 1945, **159**, 343.

Salt is what makes things taste bad when it isn't in them.*

Most people find foods unappealing and insipid without salt. Therefore, when salt restriction is indicated, the patient must be impressed with the importance of a salt-free diet and must adhere faithfully to a rigid regimen. "With the development of such preparations as Neocurtasal . . . the problem of palatability and a salty taste has been fairly well solved . . ."[1]

Neocurtasal®

"*. . . trustworthy nonsodium-containing salt substitute*"[2]

—lends the desired salty flavor to foodstuffs, and can be used in all salt-free and low sodium diets.

Constituents:

Potassium chloride, ammonium chloride, potassium formate, calcium formate, magnesium citrate and starch.

Potassium content 36%; chloride 39.3%; calcium 0.3%; magnesium 0.2%.

Neocurtasal® trademark reg. U. S. & Canada

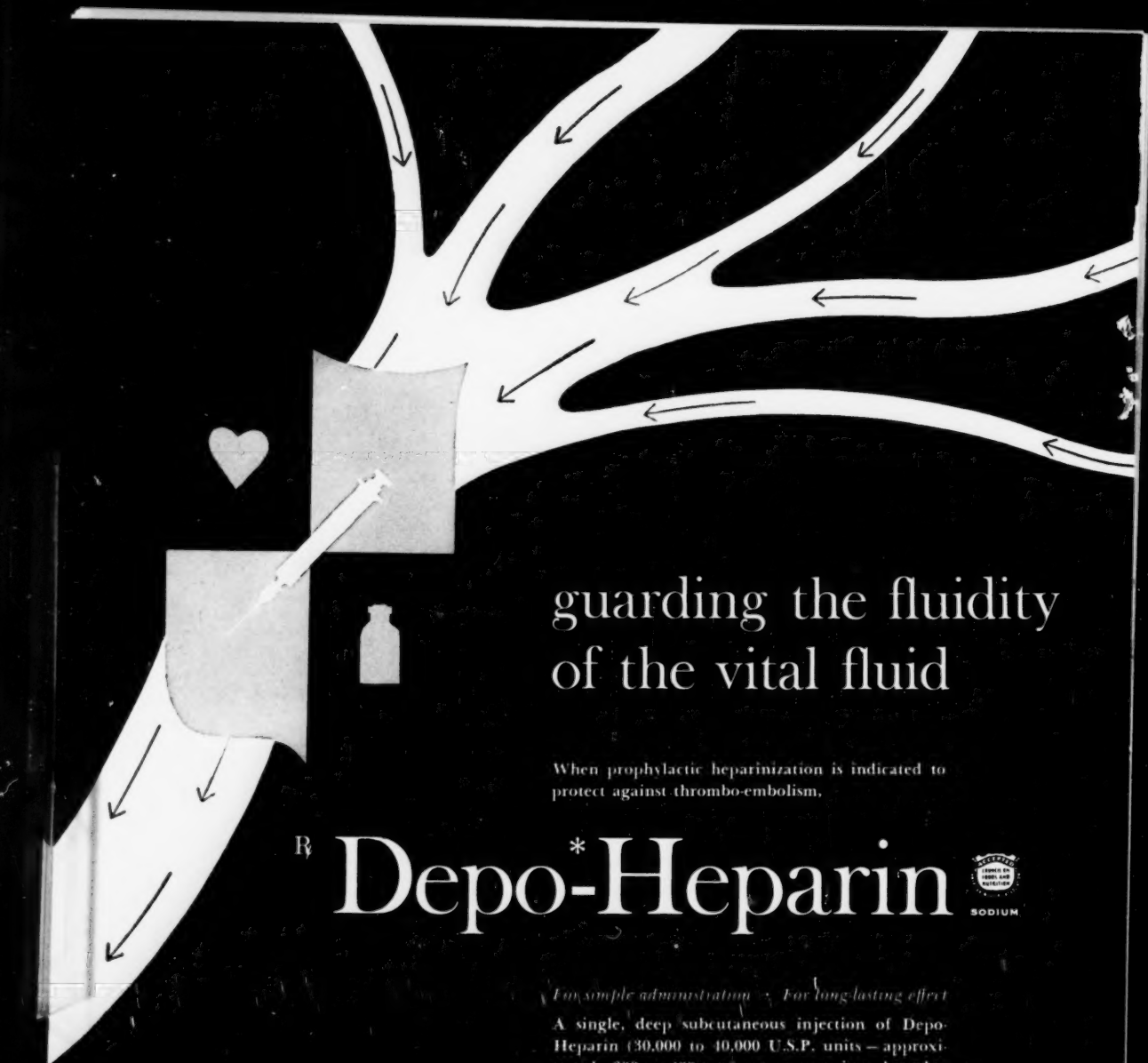
Neocurtasal looks and pours like table salt and may be used in the same manner.

Available in 2 oz. shakers and 8 oz. bottles.

Winthrop-Stearns INC.
NEW YORK 18, N. Y. WINDSOR, ONT.

1. Merryman, M. P.: The Use of the Low Sodium Diet. *South Dakota Jour. Med., & Pharm.*, 2:57, Feb., 1949.
2. Heller, E. M.: The Treatment of Essential Hypertension. *Canad. Med. Assn. Jour.*, 61:293, Sept., 1949.

*Author unidentified. From Mencken, H. L.: A New Dictionary of Quotations. New York, Alfred A. Knopf, 1942, p. 1057.



guarding the fluidity of the vital fluid

When prophylactic heparinization is indicated to protect against thrombo-embolism,

℞ Depo*-Heparin SODIUM

For simple administration — For long-lasting effect

A single, deep subcutaneous injection of Depo-Heparin (30,000 to 40,000 U.S.P. units — approximately 300 to 400 mg.) protects against thrombo-embolism for about 24 hours.

Each cc. contains:

Heparin Sodium	20,000 U.S.P. units (approx. 200 mg.)
Gelatin	180 mg.
Dextrose, Anhydrous	80 mg.
Water for Injection	q.s.

Preserved with sodium ethyl mercuri thiosalicylate 1:10,000

Supplied with sterile disposable 1 cc. cartridge syringe.

* Trademark, Reg. U. S. Pat. Off.

a product of **Upjohn** ***Research*** *for medicine . . . produced with care . . . designed for health*

THE UPJOHN COMPANY, KALAMAZOO, MICHIGAN

'AEROSPORIN'® brand

Polymyxin B

parenterally
or
orally
or
topically. . .

according to the location of any condition due to:

Pseudomonas aeruginosa (*B. pyocyaneus*), *Shigella*, and other gram-negative bacteria.

'AEROSPORIN' brand *Polymyxin B* (*Sulfate*) STERILE

500,000 Units
Equivalent to **50 mg.**
Polymyxin Standard

For intramuscular or intrathecal injection, to treat systemic or meningeal infections.

AVAILABLE TO HOSPITALS ONLY

'AEROSPORIN' brand *Polymyxin B* (*Sulfate*) STERILE

200,000 Units
Equivalent to **20 mg.**
Polymyxin Standard
For topical use only

For preparing solutions and ointments (0.1% to 0.25%) for topical use.

AVAILABLE TO HOSPITALS AND RETAIL PHARMACIES

'AEROSPORIN' brand *Polymyxin B* (*Sulfate*) COMPRESSED

500,000 Units
Equivalent to **50 mg.**
Polymyxin Standard

For oral use in enteric infections only.

AVAILABLE TO HOSPITALS AND RETAIL PHARMACIES

BURROUGHS WELLCOME & CO. (U.S.A.) INC., *Tuckahoe 7, N. Y.*

NEW... ready-to-use antibiotic combination

Combiotic*

aqueous suspension

(Penicillin and Dihydrostreptomycin)

provides in each disposable cartridge or 2 cc. dose from vial

Penicillin G Procaine . . . . . .400,000 units

Dihydrostreptomycin (as the sulfate) . . . .0.5 Gm.

Supplied:

Single injection B-D† disposable cartridge syringes; 10 cc. "drain-clear" vials containing 5 doses.

clinically preferred...

in the treatment of certain mixed infections of the urinary tract, acute gonococcal infections, in selected cases of subacute bacterial endocarditis, and in surgical prophylaxis

because of these advantages...

- no mixing or reconstitution necessary
- microparticle suspension flows easily through 22-gauge needle
- drain-clear cartridge and vial means ability to withdraw full labeled volume
- buffered for stability; maintains full potency for one year under refrigeration

also available as Combiotic P-S (dry) in 3 cc. vials containing 300,000 units Penicillin G Procaine Crystalline, 100,000 units Buffered Penicillin G Sodium Crystalline, 1 gm. Dihydrostreptomycin as the sulfate; easily prepared by addition of sterile aqueous diluent.*

*Trademark, Chas. Pfizer & Co., Inc.
†Trademark, Becton, Dickinson and Company

Antibiotic Division **Pfizer** CHAS. PFIZER & Co., INC., *Brooklyn 6, N. Y.*

LANCASTER PRESS, INC., LANCASTER, PA.